Effect of post-digestion temperature on serial CSTR biogas reactor performance

Kanokwan Boe, Dimitar Karakashev, Eric Trably, Irini Angelidaki

Effect of post digestion temperature on serial CSTR biogas reactor performance

Kanokwan Boe, Dimitar Karakashev, Eric Trably* and Irini Angelidaki**

Department of Environmental Engineering, Technical University of Denmark, Building 113, DK-2800, Kgs. Lyngby, Denmark

* INRA - LBE UR050, Laboratoire de Biotechnologies de l'Environnement, Avenue des Etangs, 11100 Narbonne, France

** Corresponding author. Phone: (+45)45251429, Fax: (+45)45932850, E-mail: ria@env.dtu.dk

Abstract

The effect of post digestion temperature on a lab-scale serial continuous-flow stirred tank reactor (CSTR) system performance was investigated. The system consisted of a main reactor operated at 55 ^{o}C with hydraulic retention time (HRT) of 15 days followed by post digestion reactors with HRT of 5.3 days. Three post digestion temperatures (55 ^{o}C, 37 ^{o}C and 15 ^{o}C) were compared in terms of biogas production, process stability, microbial community and methanogenic activity. The results showed that the post digesters operated at 55 ^{o}C, 37 ^{o}C and 15 ^{o}C gave extra biogas production of 11.7%, 8.4% and 1.2%, respectively. The post digester operated at 55 ^{o}C had the highest biogas production and was the most stable in terms of low VFA concentrations. The specific methanogenic activity tests revealed that the main reactor and the post digester operated at 55 ^{o}C and 37 ^{o}C had very active acidogens and methanogens. In contrast, very low methanogenic activity was observed at 15 ^{o}C.

Keywords: biogas; post digestion; temperature

Introduction

Anaerobic digestion of manure or other slurries is commonly performed in continuous-flow stirred tank reactors (CSTR). A single CSTR is simple to operate but less efficient compared to immobilized-cell configurations, such as upflow anaerobic sludge blanket (UASB). However, manure slurry is not suitable for direct application in the UASB reactor due to its high content of particulate matter. In CSTR, the slowly degradable fibers in manure are normally not fully utilized during the digestion (Ahring and Angelidaki, 2000). It has been reported that only 50-70% of organic matter is converted to biogas in a manure digester with typical hydraulic retention time (HRT) of 15-30 days (Hartmann et al., 2000). Moreover, a single CSTR digester can “loose” biogas production due to “short-circuit”, where parts of organic material in the feed remain in shorter duration in the reactor than the nominal retention time (Angelidaki et al., 2005). There are several reports on improving biogas production in the CSTR process, for example, by increasing hydraulic retention time (Hansen et al., 1998), co-digestion with other organic wastes (Ahring et al., 1992), or by pre-treatment of the substrate to improve degradation of recalcitrant materials (Ahring and Angelidaki, 2000; Hartmann et al., 2000). Simulation results using Anaerobic Digestion Model 1 (ADM1) (Boe et al., 2005) showed that a serial CSTR configuration consisting of one reactor (or so-called main digester) with long retention time and another reactor (or so-called post digester) with short retention time could improve biogas production and achieve better effluent quality in terms of VFA (volatile fatty acids) concentration compared to a single CSTR reactor. Once the main digester has sufficient volume to maintain stable operation, extra biogas production could be obtained by post digestion (Angelidaki et al., 2005). Full-scale biogas reactors are commonly connected to effluent storage tanks

to store digested manure before recycling to agricultural farmlands. These effluent storage tanks (known also as post-digesters) have normally covered their top for collection of the extra biogas that might be produced in post digesters. However, these tanks have often no temperature control installed, which can affect the microbial activity and composition and consequently the biogas production. It is well-known that one of the main factors affecting anaerobic digestion process is the temperature (Hashimoto, 1983). Over the last decades, the effect of temperature on biogas production, effluent quality (in terms of VFA concentrations) and process stability have been widely investigated (Harris and Dague, 1993; Kugelman and Guida, 1989). Some studies showed better performances of thermophilic anaerobic digestion compared to mesophilic one as indicated by the lower VFA concentrations and higher biogas production (Kim et al., 2002; Mackie and Bryant, 1995). In general, higher process temperatures enhance microbial activities, resulting in higher biogas production. With regards to microbial community structure, thermophilic conditions result in lower microbial diversity compared to mesophilic conditions (Karakashev et al., 2005; Sekiguchi et al., 1998). Additionally, the thermophilic reactors are more susceptible to ammonia inhibition compared to the mesophilic reactors. Indeed, high temperatures increase free ammonia concentrations which negatively affect the anaerobic digester performances (Angelidaki and Ahring, 1994). Furthermore, increasing temperatures also increase energy costs for heating the post digesters. Therefore, the benefits of increasing post digestion temperatures should be thoroughly investigated.

The objective of this study was to investigate the effect of temperatures on the post digester performances. Three post digesters operated at temperatures of 55 °C, 37 °C and 15 °C were compared in terms of biogas production, effluent quality and process

stability. The structure of the microbial communities and methanogenic activity were also determined.

Materials and Methods

Substrate characteristics

Substrate fed to the main reactor was cow and pig manure mixed with approx. 25 % of industrial wastes (mainly dairy and slaughterhouse wastes). The substrate was obtained in one batch from a full-scale biogas plant (Lemvig, Denmark). The substrate was blended to ensure homogeneity, and then stored at -20 °C for the whole period of experiment. The frozen substrate was thawed and kept at 4 °C for 2-3 days before use. Substrate characteristics are shown in Table 1.

Reactor setup and operations

The experiment was carried out in a serial configuration of CSTR digesters (Figure 1). The main digester (referred as R0) was made of a 9 L reactor with 7.2 L working volume and a hydraulic retention time (HRT) of 15 days. Reactor R0 was built from double glass cylinder fitted with stainless steel plates on 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. The operating temperature in reactor R0 was maintained at 55 °C by circulating hot water through the space between the reactor glass walls. Reactor R0 was connected to three post digesters as shown in Figure 1. The three post digesters were operated in parallel with operating temperatures of 55 °C (referred as R55), 37 °C (referred as R37), and 15 °C (referred as R15), respectively. Each post digester was made of a 1 L glass reactor with 800 mL

working volume and HRT of 5.3 days, which was in the range of typical HRT of post digester used in the Danish full-scale biogas plants. The operating temperatures in the post digesters were controlled by circulating hot water (for R55 and R37) or cooling liquid (for R15) through a water jacket surrounding the reactors.

The amount of 120 mL substrate was fed into the main reactor four times per day using peristaltic pump with timer control. Each post digester was fed with 150 mL of the effluent from the main reactor once per day by manually start a peristaltic pump. Rest of the effluent from the main reactor (less than 30 mL per day) was collected in the effluent bottle of the main digester. All reactors were continuously stirred throughout the experiment by motor mixer in the main reactor and by magnetic stirrers in the post digesters. Operating pressure of all reactors was around 1 atm. The slight overpressure from the produced biogas and the influent inside the reactors pushed out the effluents from liquid surface through the effluent tubes on top of the reactors. The effluents were collected in the effluent bottles. The effluent bottles were connected with gas meters that registered the produced biogas production.

Analytical methods

The biogas production was measured by an automated displacement gas metering system with a 100 mL reversible cycle and registration (Angelidaki et al., 1992). The water used in gas meter was acidified to pH 3 by HCl and NaCl was added to prevent gas dissolution. Methane content of the biogas was measured by a gas chromatograph (Mikrolab, Århus) equipped with thermal conductivity detector and a glass column, 6ft x 3mm I.D., packed with Poropack Q (10/80). The injector, detector and oven temperature were isothermal at 55 ºC. The carrier gas was Helium with flow rate 40

mL/min. Theoretical biogas production was calculated according to Angelidaki and Sanders (2004). Ammonium and total nitrogen, pH, total solids (TS), and volatile solids (VS) were determined according to standard methods (Greenberg et al., 1992). Free ammonia was estimated as described in Angelidaki et al. (1999). VFA was measured using a gas chromatograph (GC) HP 5890 Series II equipped with flame ionization detector and a FFAP fused-silica capillary column, 30m x 0.53mm I.D., film thickness 1.5 µm. Nitrogen was used as a carrier gas. The oven temperature was initially set at 70 ^{o}C and then increasing 10 degrees/min. to 190 ^{o}C and kept at final temperature for 3 min. The injection port and detector temperature were 150 ^{o}C and 200 ^{o}C, respectively.

Pulse load tests

At day 94, a lipid pulse load was introduced to study the effect on the process stability of the different setups. The pulse load was added by directly injecting 219 g of rapeseed oil, which corresponding to 14.6 times of the normal VS load, into the main reactor R0.

Specific methanogenic activity (SMA) tests

At day 62, batch experiments for measuring specific methanogenic activity (SMA) on a specific substrate were carried out (Angelidaki and Schmidt, 2002). 40 mL basal anaerobic (BA) medium prepared as described in Karakashev et al., (2005) was dispensed anaerobically in 100 mL serum bottles. The media was supplemented with different substrates- acetate (20 mM), propionate (10 mM), butyrate (10 mM), hydrogen/carbon dioxide (50/50) under 1 atm, and glucose (10 mM). After addition of vitamin solution and $Na_2S.9H_20$ as a reducing agent the medium was inoculated with 10 mL fresh samples from each reactor and incubated in respective temperature of

inoculums. Bottles with BA medium and inocula only but without substrates were used as controls (blanks). All the tests were prepared in duplicates. Methane production and VFA levels were monitored closely for the first 10 days and thereafter for 50 days.

Microbial community composition

Fluorescent *in situ* hybridization (FISH) method of Hugenholtz et al. (2001) was used to assess main phylogenetic groups of methanogens, i.e. *Methanomicrobiales, Methanococcales, Methanobacteriales Methanosaetaceae and Methanosarcinaceae,* as previously described by Karakashev et al. (2005).

Polymerase chain reaction-temperature gradient gel electrophoresis (PCR-TGGE) was used to determine microbial community profiles of the reactors according to Muyzer and Smalla (1998).

Results

Biogas production, VFA concentrations and pH from all reactors are presented in Figure 2. The experiment was carried out for 111 days and steady state was obtained after day 45 when VFA and biogas production had reached relatively stable values (daily variations lower than 14% in the main reactor). The process parameters during steady state are summarized in Table 1. Methane content was found similar in all reactors around 70%. During steady state average biogas production in reactors R0, R55, R37 and R15 were of 24.6, 2.9, 2.1 and 0.3 mL biogas/mL feed, respectively (Figure 2a). In comparison, the theoretical biogas production resulting from full decomposition of the organic matter in raw manure would have been approximately 40 mL-biogas/mL-feed

(0.5 L-CH_4/gVS). The additional biogas obtained from the post digesters were 11.7%, 8.4% and 1.2% for R55, R37 and for R15 respectively.

During steady state, total VFA concentrations in reactor R0, R37 and R15 were similar (20 mM in average), while reactor R55 had significantly lower VFA concentration (4 mM). This corresponded to the low %TS and %VS in the reactor R55 compared to other reactors. Reactor R37 and R15 seemed to have biomass accumulation due to lower removal of organic matters. Acetate and propionate were predominant VFA in all reactors. Total VFA concentration in reactor R0 followed the trend of total VFA in the feed, and total VFA concentrations in reactor R37 and R15 also followed the trend of VFA in reactor R0 (Figure 2b). In contrast, the VFA concentrations in reactor R55 were constantly low, almost independent from the VFA concentration in R0. The pH values in all reactors were relatively stable in the range of 7.7-8.1. Levels of ammonium nitrogen in all reactors were similar within a range of 3.3-3.8 g-N/L (Table 1). The corresponding calculated free ammonia was ranged from 0.13 to 1.1 g-N/L.

After adding a pulse load of 219 g rapeseed oil into reactor R0 on day 94, a double increase in biogas production and subsequent increased VFA in reactor R0 were observed. Similar responses were noticed in all post digesters. Nevertheless, reactor R55 showed smaller increase in VFA concentration compared to the increase observed in R37 and R15. Indeed, the effect of pulse load was more pronounced in R15 and R37. In all reactors, pH did not change significantly after the lipid pulse load (Figure 2).

In regards to microbial activities, specific methanogenic activity (SMA) tests revealed that the degradation rate of the tested substrates increased with the increase of temperatures from 15 °C to 55 °C (Table 2). Higher degradation rates of acetate, H_2/CO_2, butyrate and propionate were observed in R0 and R55 compared to R37 and

R15. A higher methanogenic activity from acetate was noticed in R37 than R55. No or very low degradation was noticed for all the tested substrates in R15. These results were supported by FISH and PCR-TGGE analysis of microbial community composition in all reactors (pictures not shown). According to the FISH results, both *Bacteria* and *Archaea* (especially *Methanosaetaceae*) were more abundant in R0, R55 and R37 compared to R15, where the relative abundance was in order of R0>R55>R37. Regarding the PCR-TGGE results, the microbial community profiles at day 62 and day 93 were different for both abundance and community structure. From the bacterial and archaeal community profiles, the relative abundances of microorganisms were compared based on the intensity of DNA bands, and summarized in Table 3.

Profiles of bacterial community showed four well separated bands representing four different phylogenetic groups of dominant *Bacteria* in the samples. At day 62, band 1 existed only in reactor R0 and R15. Band 3 was more abundant in reactor R37 and R55, while band 4 was more abundant in R0 and R15. Bacterial diversity in reactors R37 and R55 was significantly lower compared to R0, while the bacterial profile in reactor R15 was similar compared to R0. At day 93, band 1 disappeared from reactor R0 and R15, and band 3 disappeared from all reactors. Comparing day 93 to day 62, relative abundance of band 2 in reactors R0, R55 and R15 increased with 27 %, 32 % and 35 %, respectively. For reactor R37, band 4 abundance increased with 38 % compared to day 62 but the abundance of band 2 was relatively constant with time.

Archaea community profile showed four well separated bands representing four different phylogenetic groups of dominant *Archaea*. At day 62, band 2 was lacked from R37. The diversity of *Archaea* in reactor R0 and R55 did not change significantly with time. However, a change in abundance was observed. Band 2 in reactor R0 and R55 was

enriched at day 93 compared to day 62. In contrast, at day 93 band 2 disappeared from reactor R15 and appeared in R37.

Discussion

Many researchers have reported strong effect of temperature on the microorganisms where lowering the operational temperature led to a decrease in the maximum specific growth and substrate utilization rates. Under psychrophilic conditions (10-15 ^{o}C), chemical and biological reactions proceed much slower than under mesophilic conditions (30-40 ^{o}C) (Lettinga et al., 2001). The results from the present study also showed that operating temperature in the post digesters had significant effect on biogas production, methanogenic activities and microbial community. The higher additional biogas production obtained from R55 and R37 compared to R15 (Figure 2a) suggested that operating temperature in the post digesters should be maintained at temperature higher than 15 °C. Additional biogas production in post digesters came from residual VFA and residual organic matter present in the effluent of the main reactor (R0 effluent). If the biogas production in post digesters only came from residual VFA decomposition, then the amount of biogas produced should be close to the value of biogas equivalent from VFA in R0 effluent, which was 1.1 mL-biogas/mL-feed (Figure 3a). However, it was observed that the amount of biogas obtained in reactors R55 and R37 were 2.9 and 2.1 mL-biogas/mL-feed, respectively, which were higher than the biogas equivalent from VFA in R0 effluent. This implies that VFA were also produced in reactor R55 and R37 from acidogenesis of residual organic matters. Comparatively, reactor R15 had very low biogas production and VFA production. From Figure 3b, the combination of R0 with R55 could obtain biogas production of 27.5 mL-biogas/mL-

feed, which corresponds to around 70% of the theoretical biogas potential in the feed (40 mL-biogas/mL-feed), i.e. to approx. 70% utilization of the organic matter in manure. The combination of R0 with R37 and R15 could obtain 67% and 62% of the theoretical biogas potential in the feed, respectively. This showed that maintaining high temperature in the post digester could increase biomass utilization in the system. Moreover, R55 and R37 were more efficient than R15 to recover the biogas yield from VFA in R0 effluent during overload. As it was seen that adding lipid pulse at day 94 increased biogas production and VFA concentration in reactor R0 with subsequently increased biogas production in R55 and R37 compared to R15.

The low methane yield in R15 revealed that methanogenesis was inactive, and the similar VFA concentrations between R0 and R15 meant that acidogenesis occurred at very low extent. Moreover, compared to R55 and R37, reactor R15 was more dependent on R0 as its variation in acetate concentration strongly followed the trend of R0 (Figure 2b). The constant low VFA concentrations and high biogas production in R55 confirmed its stability and independence from the variations in R0, although the high free ammonia level in R55 (1.1 g-N/L) could be inhibitory according to the threshold limit reported from Hansen et al. (1998). The high concentrations of free ammonia in reactors R0 and R55 were mainly due to high operating temperature (Angelidaki and Ahring, 1994). Moreover, the total ammonia concentration in all reactors were in the range of 3-4 g-N/L. It was previously shown that the total ammonia concentration up to 4 g-N/L did not result in process inhibition both at thermophilic and mesophilic conditions (Angelidaki et al., 2005).

The results from SMA tests showed a good correlation to the results from reactor operational parameters measured at steady state. The high initial degradation

rates (Table 2) showed for all tested substrates in reactor R0, R55 and R37 suggested a high activity of all microorganisms (both acidogens and methanogens). Moreover, glucose degradation in reactor R37 was as efficient as that in R55, indicating that acidogenesis were equally active in these two reactors. The higher aceticlastic methanogenic activity from acetate in R37 compared to R55 suggested that aceticlastic methanogens were more active at mesophilic conditions than at thermophilic. This could be due to the higher methanogenic diversity in mesophilic compared to thermophilic processes (Sekiguchi et al, 1998). Some aceticlastic methanogenic species present in R0 could grow better in R37 than in R55 under the same post digester HRT. The low activity on glucose in R15 suggested that acidogenesis from hexoses was low. Very low methane production from acetate and H_2/CO_2 and no consumption of butyrate, propionate in R15 suggested that both acetogens and hydrogenotrophic methanogens were inactive.

The FISH results showing high abundance of different phylogenetic groups of *Bacteria* and *Archaea* in R0, R37 and R55 indicated that acidogens and methanogens were active in these reactors. This is in good agreement with the results from reactor operation and SMA tests. Moreover, the FISH results showing low microbial abundance in R15 suggested that the microorganisms might be metabolically inactive, resulting in low content of ribosomal RNA which could be detected by FISH technique. This observation correlated to almost no biogas production and very low activity in the SMA test at low temperature. Nevertheless, we believe that the microorganisms were not washed out from the reactor R15 although they were inactive, since R15 was running at very short retention time (HRT 5.3 days) and always received the fresh effluent from R0 which contained large amount of active microorganisms.

Results obtained by PCR - TGGE analysis also showed that the reactor temperature affected microbial community structures. At day 62, the community profile of both *Bacteria* and *Archaea* in reactor R15 was very similar to R0. This was due to the fact that lower temperature resulted in lower specific growth rate (Kevbrina et al., 2001), thus, the microbial population remained unchanged. At 55 ^{o}C and 37 ^{o}C, the microorganisms were more active so the enrichment of some methanogenic group in the post digester compared to reactor R0 could be observed.

Although the degradation rate is very slow at psychrophilic temperature, it has been reported that stable methanogenesis can still be achieved if the microorganisms has been long-term adapted to the low temperature (> 4 months) (Kettunen and Rintala, 1997; Lettinga et al., 1999). If this is the case, the post digestion at 15 ^{o}C might be possible if R15 is large enough to build up its own adapted culture. However, in this experiment the post digesters strongly relied on the microorganisms in R0 effluent which were thermophiles. The microorganisms coming into R15 were, in some degrees, exposed to temperature shock and became inactive.

Results obtained in this study showed that the post digestion at 37 ^{o}C and 55 ^{o}C provided extra biogas yield which could give reasonable compromise between the cost of heating and the benefit from additional biogas. However, the cost of heating is strongly depending on the quality of reactor insulation. Before applying this idea in the full-scale application, an economic evaluation should be done comparing the investment costs for insulation of the existing post digesters, the operational costs for heating, and the amount of additional biogas energy expected from the post digesters.

Another benefit of using high temperature in post digestion is that the effluent from the biogas plants would contain less residual organics which will minimize the

methane emission from degradation of the residual organics during transportation back to the farmers and during storage period before manure spreading on agricultural land.

Conclusion

Results obtained in this study showed that the temperature could affect biogas production and microbial ecology in the post digesters. When the main biogas digester is running at thermophilic temperature, the operating temperature of 15 ^{o}C in the post digesters is very inefficient as the bacteria become inactive as they were unadapted to low temperature. The post digester operated at 37 °C could obtain similar biogas production as at 55 °C. The specific methanogenic activity tests showed that the main reactor had highest microbial activities, followed by the post digester with 55 °C and 37 °C. Moreover, post digestion temperature of 55 ^{o}C could also provide the effluent with low VFA, which means lower potential of smell when recycling on farmland. Thus, it is recommended that the temperature in the post digester should be kept as close as possible to the temperature of the main digester to maintain the bacterial activities.

Acknowledgements

This work was supported by the Ph.D. scholarship from the Department of Environmental Engineering, Technical University of Denmark, and the Danish Energy Agency, Project EFP-04. The authors would like to acknowledge our former master students, Tore Linde and Jacob Kragh Andersen, for their contributions during the experiments in this study.

References

Ahring, B. K., Angelidaki, I. and Johansen, K. (1992) Anaerobic treatment of manure together with industrial waste. Water Science and Technology 25, 311-318.

Ahring, B. K. and Angelidaki, I. (2000) Methods for increasing the biogas potential from the recalcitrant organic matter contained in manure. Water Science and Technology 41, 189-194.

Angelidaki, I. and Ahring, B. K. (1994) Anaerobic thermophilic digestion of manure at different ammonia loads: effect of temperature. Water Research 28, 27-731.

Angelidaki, I., Boe, K. and Ellegaard, L. (2005) Effect of operating conditions and reactor configuration on efficiency of full-scale biogas plants. Water Science and Technology 52, 189-194.

Angelidaki, I., Ellegaard, L. and Ahring, B. K. (1992) Compact automated displacement gas metering system for measurement of low gas rates from laboratory fermentors. Biotechnology and Bioengineering 39, 351-353.

Angelidaki, I., Ellegaard, L. and Ahring, B. K. (1999) A comprehensive model of anaerobic bioconversion of complex substrates to biogas. Biotechnology and Bioengineering 63, 363-372.

Angelidaki, I. and Schmidt, J. E. (2002) Activity test for the three reactors in Green Farm Energy, Institute of Environment & Resources, Technical University of Denmark.

Angelidaki, I. and Sanders, W. (2004) Assessment of the anaerobic biodegradability of macropollutants. Reviews in Environmental Science and Biotechnology 3, 117-129.

Boe, K., Batstone, D. J. and Angelidaki, I. (2005) Optimization of serial-CSTR biogas reactors using modeling by ADM1. p.219-221, In: Proceedings of the First International Workshop on the IWA Anaerobic Digestion Model No.1 (ADM1), 2-4 September 2005, Lyngby, Denmark

Greenberg, A. E., Clesceri, L. S. and Eaton, A. D. (1992) Standard Methods for the Examination of Water and Wastewater, 18th ed., APHA, AWWA, WPCF, Washington, DC.

Hansen, K. H., Angelidaki, I. and Ahring, B. K. (1998) Anaerobic digestion of swine manure: Inhibition by ammonia. Water Research 32, 5-12.

Harris, W. L. and Dague, R. R. (1993) Comparative performance of anaerobic filters at mesophilic and thermophilic temperatures. Water Environment Research 65, 764-771.

Hartmann, H., Angelidaki, I. and Ahring B.K. (2000) Increase of anaerobic degradation of particulate organic matter in full-scale biogas plants by mechanical maceration. Water Science and Technology 41, 145-153.

Hashimoto, A. G. (1983) Conversion of straw-manure mixtures to methane at mesophilic and thermophilic temperatures. Biotechnology and Bioengineering 25, 185-200.

Hugenholtz, P., Tyson, G. W. and Blackall, L. L. (2001) Design and evaluation of 16S rRNA-targeted oligonucleotide probes for fluorescence *in situ* hybridization, p.29-42, In: Aquino de Muro, M. & Rapley, R. (eds.) Gene Probes: Principles and Protocols, Humana, London.

Karakashev, D., Batstone, D. J. and Angelidaki, I. (2005) Influence of environmental conditions on methanogenic compositions in anaerobic biogas reactors. Applied and Environmental Microbiology 71, 331-338.

Kettunen, R. S. and Rintala, J. A. (1997) The effect of low temperature (5-29 °C) and adaptation on the methanogenic activity of biomass. Applied Microbiology and Biotechnology 48, 570-576.

Kevbrina, M. V., Okhapkina, A. A., Akhlynin, D. S., Kravchenko, I. K., Nozhevnikova, A. N. and Galchenko, V. F. (2001) Growth of mesophilic methanotrophs at low temperatures. Microbiology 70, 384-391.

Kim, M., Ahn, Y. H. and Speece, R. E. (2002) Comparative process stability and efficiency of anaerobic digestion; mesophilic vs. thermophilic. Water Research 36, 4369-4385.

Kugelman, I. J. and Guida, V. G. (1989) Comparative evaluation of mesophilic and thermophilic anaerobic digestion. EPA/ 600/S2-89/001.

Lettinga, G., Rebac, S., Parshina, S., Nozhevnikova, A., Van Lier, J. and Stams, A. J. M. (1999) High-rate anaerobic treatment of wastewater at low temperatures. Applied and Environmental Microbiology 65, 1696-1702.

Lettinga, G., Rebac, S., and Zeeman, G. (2001) Challenge of psychrophilic anaerobic wastewater treatment. Trends in Biotechnology 19, 363-370.

Mackie, R. I. and Bryant, M. P. (1995) Anaerobic digestion of cattle waste at mesophilic and thermophilic temperatures. Applied Microbiology and Biotechnology 43, 346-350.

Muyzer, G. and Smalla, K. (1998) Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. Antonie van Leeuwenhoek 73, 127-141.

Sekiguchi, Y., Kamagata, Y., .Syutsubo, K., Ohashi, A., Harada, H. and Nakamura, K. (1998) Phylogenetic diversity of mesophilic and thermophilic granular sludges determined by 16S rRNA gene analyses. Microbiology 144, 2655-2665.

List of Figures

List of Tables

Table 1. Average reactor parameters over steady state period (day 45-70) and main substrate characteristics.

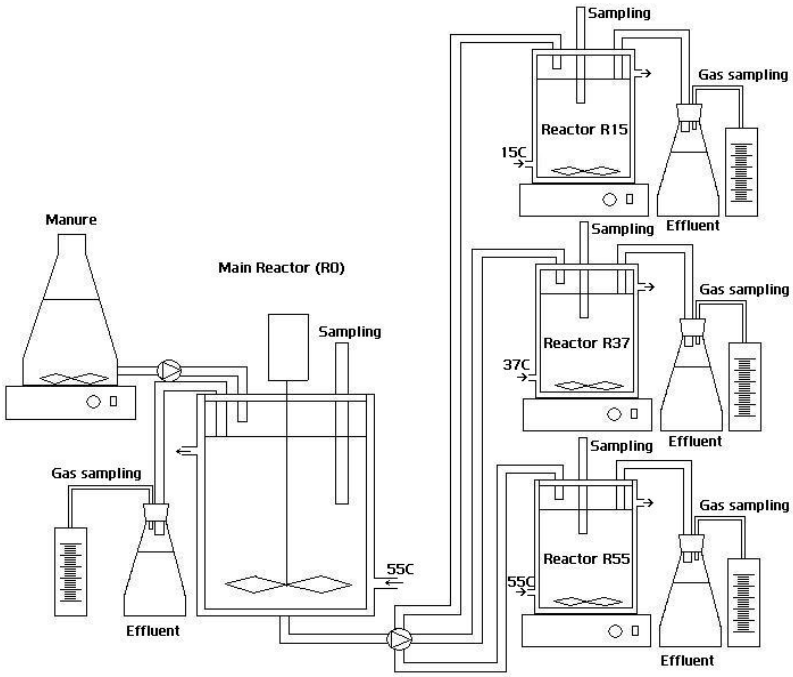
Parameters	Substrate	R0	R55	R37	R15
TS (%)	8.21	5.50	5.23	5.88	6.28
VS (%)	4.78	2.52	2.39	2.74	2.92
Total-N (g-N/L)	4.62	4.7	4.7	4.8	4.8
Ammonium-N (g-N/L)	3.26	3.3	3.6	3.8	3.3
Free ammonia (g-N/L)	0.01	0.85	1.10	0.22	0.13
Lipids (g/L)	5	<0.01	<0.01	<0.01	<0.01
pH	6.87	7.89	7.87	7.72	8.09
Total VFA (mM)	259.8	22.8	4.0	19.5	22.7
Acetate (mM)	185.1	15.8	3.4	12.3	15.3
Propionate (mM)	41.2	5.8	0.4	6.1	5.5
Iso-butyrate (mM)	6.3	0.5	0.04	0.5	0.7
Butyrate (mM)	15.9	0.2	0.02	0.1	0.3
Iso-valerate (mM)	6.2	0.3	0.05	0.3	0.7
Valerate (mM)	5.2	0.1	0.02	0.1	0.1
Biogas Production (mL-biogas/mL-feed)		24.6	2.9	2.1	0.3
Extra biogas production (%) compared to R0		-	11.7	8.4	1.2
Methane (%) in biogas		69.9	68.8	77.9	70.1
Extra methane production (%) compared to R0		-	11.6	9.3	1.2

Table 2. Microbial activity on different substrates.

Substrate	Activity unit	R0	R55	R37	R15
H_2/CO_2	mL CH_4/(gVS.hr)	0.27	0.24	0.16	0.008
Acetate	mL CH_4/(gVS.hr)	0.50	0.46	0.48	0.005
Propionate	mM Pr/day	0.90	0.70	0.50	-
Butyrate	mM But/day	3.10	1.70	1.30	-
Glucose	mmole CH_4-equivalent/day	0.47	0.40	0.40	0.043

Table 3. Percentage relative abundance of *Bacteria* and *Archaea* in each sample.

Domain	Bands	Day 62				Day 93			
		R0	**R55**	**R37**	**R15**	**R0**	**R55**	**R37**	**R15**
Bacteria	1	12	0	0	13	0	0	0	0
	2	17	25	25	19	44	57	27	54
	3	23	38	40	21	0	0	0	0
	4	48	37	35	47	56	43	73	46
	% Total	100	100	100	100	100	100	100	100
Archaea	1	19	28	29	23	18	21	24	37
	2	30	13	0	20	39	28	20	0
	3	28	22	31	32	24	24	31	26
	4	23	37	40	25	19	27	25	37
	% Total	100	100	100	100	100	100	100	100



1 Figure 1. **Reactor setup for the experiment**

2

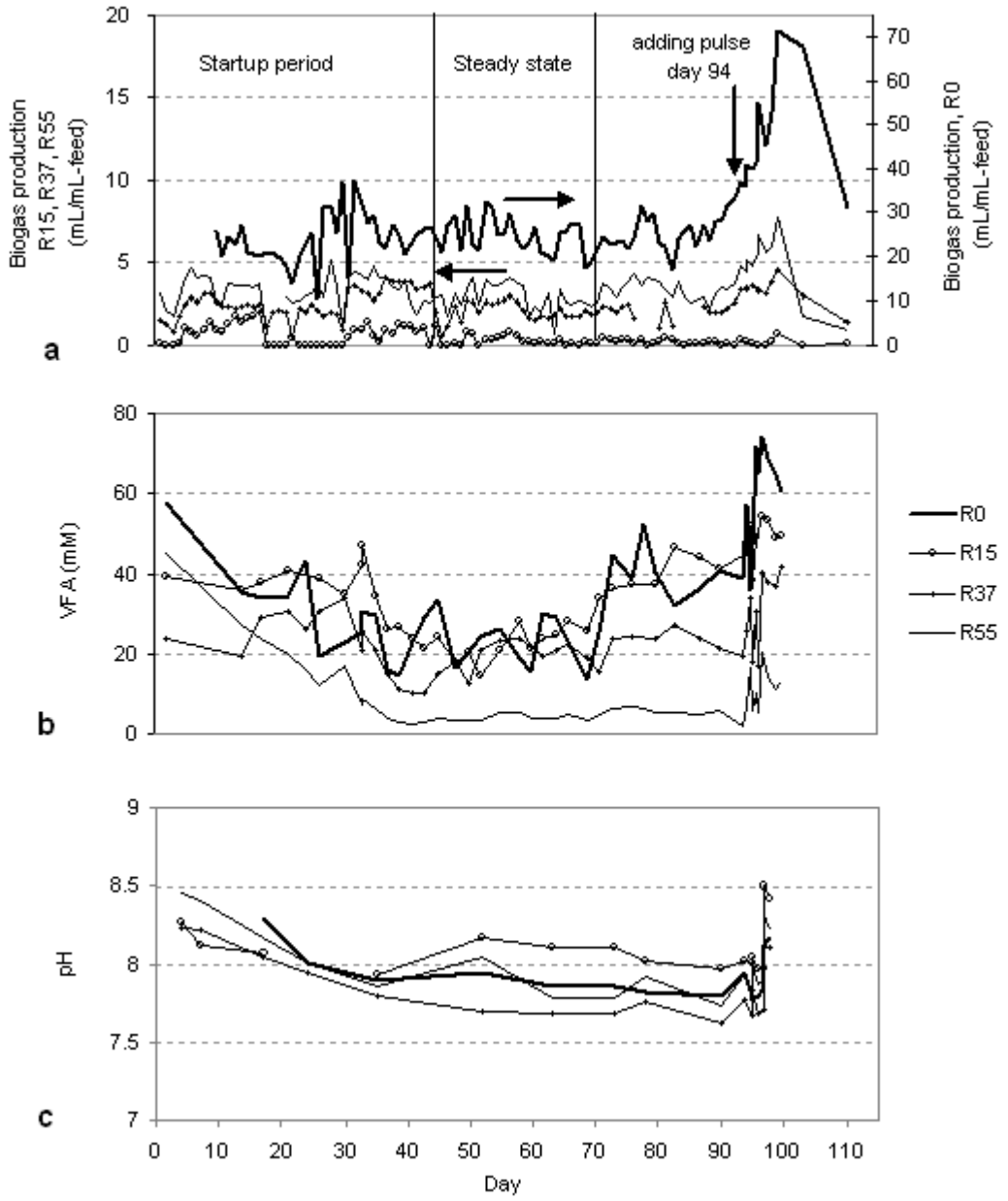


Figure 2 Experimental results: (a) Biogas production, (b) VFA concentration, (c) pH values

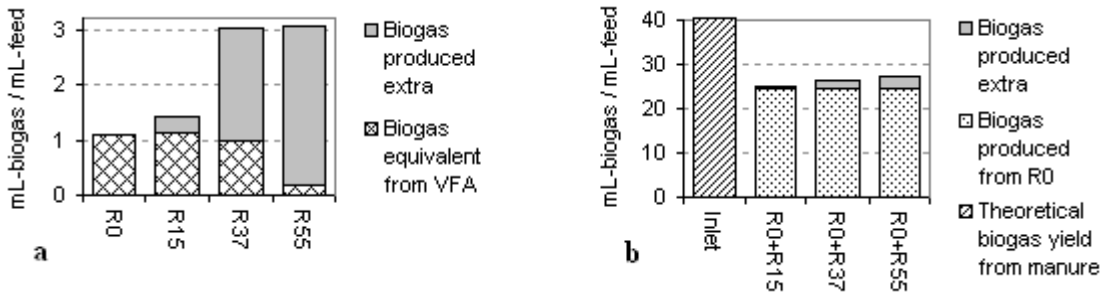


Figure 3. Comparison of the actual and theoretical biogas yield; (3a) Actual biogas production obtained in post digesters compared to biogas potential from VFA in R0 effluent, (3b) Actual biogas production from R0 and post digesters compared to theoretical biogas yield from manure