Thermophilic anaerobic digestion in compact systems: investigations by modern microbiological techniques and mathematical simulation

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Abstract Thermophilic anaerobic digestion in compact systems can be an economical and ecological reasonable decentralised process technique, especially for rural areas. Thermophilic process conditions are important for a sufficient removal of pathogens. The high energy demand, however, can make such systems unfavourable in terms of energy costs. This is the case when low concentrated wastewater is treated or the system is operated at low ambient temperatures. In this paper we present experimental results of a compact thermophilic anaerobic system obtained with fluorescent *in situ* hybridisation (FISH) analysis and mathematical simulation. The system was operated with faecal sludge for a period of 135 days and with a model substrate consisting of forage and cellulose for a period of 60 days. The change in the microbial community due to the two different substrates treated could be well observed by the FISH analysis. The Anaerobic Digestion Model no. 1 (ADM1) was used to evaluate system performance at different temperature conditions. The model was extended to contribute to decreased methanogenic activity at lower temperatures and was used to calculate energy production. A model was developed to calculate the major parts of energy consumed by the digester itself at different temperature conditions. It was demonstrated by the simulation study that a reduction of the process temperature can lead to higher net energy yield. The simulation study additionally showed that the effect of temperature on the energy yield is higher when a substrate is treated with high protein content.

Keywords ADM1; decentralized sanitation and reuse; energy; modelling; thermophilic anaerobic digestion

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Introduction

Bacteria involved in the anaerobic digestion process have a defined temperature range in which they are most productive in terms of maximum growth rate or substrate degradation rate. In addition, different groups of bacteria have different temperature optima. Microorganisms with various temperature characteristics are referred to as: psychrophiles with low temperature optima (15–20°C), mesophiles with mid-range temperature optima (25–40°C), thermophiles with high temperature optima (45–80°C) and hyperthermophiles with very high temperature optima (>80°C). In technical systems, the most commonly used process is mesophilic digestion operated at 35°C, followed by thermophilic digestion operated at 55°C.

The main advantages gained by thermophilic digestion are increased reaction rates allowing lower retention times and increased destruction of pathogenic organisms compared to mesophilic digestion (Buhr and Andrews, 1977). Lower retention times will decrease capital costs as smaller systems can be used. The sanitising effect, which was found to be a combined effect of temperature and the anaerobic environment (Lund *et al.*, 1996), can lead to an effluent reusable in agriculture. These advantages are of most importance when anaerobic digestion is performed in compact systems and the effluent is intended to be used for irrigation. However, it is often reported in the literature that

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thermophilic anaerobic digestion has a lower degree of stability leading to higher effluent concentrations of volatile fatty acids (Wiegant, 1986). In other studies (Ahring, 1994), thermophilic digestion was shown to be as stable as mesophilic digestion, when special precautions are applied during the start-up period. Nevertheless, a main drawback may be seen in the high energy requirement for system operation. Energy is needed to maintain process temperature, compensate for irradiation loss and to heat the substrate to the operating temperature. When ambient temperature and organic load is high, energy requirements can be supplied by the gas produced. However, the digestion of substrates with low solids concentration and/or the operation during cold periods makes the thermophilic process unfavourable in terms of energy costs.

In a previous study (Lübken *et al.*, 2007) it has been shown that a decentralised thermophilic anaerobic process technique could reach sufficient removal efficiencies of faecal coliforms and intestinal enterococci. As a result the effluent can be used for irrigation. Sufficient removal efficiencies could be ensured by a decrease of temperature down to 45°C if a hydraulic retention time (HRT) of at least five days is applied. This resulted in the question of whether energy could be saved during cold periods if a lowered temperature (but still in the range of thermophilic anaerobic digestion) is applied.

A simulation study using the Anaerobic Digestion Model no.1 (ADM1; Batstone *et al.*, 2002) was carried out to evaluate the effect of decreased temperature on energy production. ADM1 was chosen as it represents a generally applicable model for mathematical simulation of the anaerobic degradation of different types of organic substrates. The model provides a broad set of kinetic parameters for 35°C (mesophilic) and 55°C (thermophilic). However, microbial activity decreases if temperature differs from the optimum. In this study, ADM1 was extended to account for decreased microbial activity at decreased temperatures.

Parallel, fluorescent *in situ* hybridisation (FISH) was used as molecular technique to study the microbiological population of the anaerobic system. FISH has been widely used as a method for identification and quantification of microbial communities containing hydrolytic and methanogenic consortia (Raskin *et al.*, 1994; Domingues *et al.*, 2002). Owing to the application of the fluorescent dye-labelled probes directly on environmental samples the identification and quantification of microorganisms at different levels of the phylogenetic depth can be obtained. Uncultured hydrolytic and methanogenic bacteria can be identified and quantified on tested systems. The *in situ* hybridisation also provides an estimation of the growth rates of the microbial population, since the ribosomal content varies with the growth rate (Walner *et al.*, 1993).

Material and methods

Pilot plant and substrate characteristics

The thermophilic system had a total volume of 500 L, which was separated into an outer chamber for hydrolysis and an inner part for methanogenesis. The inner part consisted of a fixed bed and was separated by a sieve from the hydrolysis chamber. A detailed description is given in Lübken *et al.* (2007). For a period of 135 days, the system was fed with faecal sludge as the sole substrate. Total solids were in the range of 0.15–0.25% and the average volatile solids content was 91%. Owing to the low TS content, a hydraulic load of 200 L per day had to be applied to achieve a minimum organic loading rate of 0.55 kg-VS/($m^3 \cdot d$). To investigate reactor performance at high total solids concentrations a model substrate was used consisting of forage and cellulose. Powder cellulose was used to imitate structural materials (raw fibres), forage to imitate a protein-rich substrate. The model substrate was mixed in a ratio of 75% forage and 25% cellulose with influent of a wastewater treatment plant (WWTP Sulzbach-Rosenberg, Germany) to

regulate a TS content of 7%. Owing to the high protein content (25% of TS) the treatment of the model substrate revealed insights into the possible application of food waste as co-substrate. The system was operated with the model substrate for a period of 60 days. Average organic loading rate was 3 kg-VS/(m^3· d) and the HRT was reduced to a constant 20 days.

Sampling and FISH analysis

The samples for the *in situ* hybridisation were collected from the outer and the inner chamber of the reactor; for the hydrolytic and methanogenic phase, respectively. After sampling, the reactor samples were fixed separately applying two methods: (a) with 4% paraformaldehyde solution and (b) with ethanol in 1:1 ratio (v/v), both as described by Amann *et al.* (1990). In general, paraformaldehyde fixation is most suitable for Gram-negative bacteria and for Gram-positive bacteria the fixation with ethanol is recommended due to their more complex membranes (Roller *et al.*, 1994). The storage of the cell suspension took place at −20°C.

The hybridisation was performed according to Raskin *et al.* (1994). Fixed cells (about 5–10 μL) were applied directly to wells on gelatin-coated slides (double-row shifted, Co. Paul Marienfeld, Bad Mergentheim). Samples were immobilised on glass slides by air-drying, followed by dehydration using 50, 80 and 100% ethanol series. All the hybridisations with the selected probes [EUB338, Amann *et al.*, 1990; EUB338II/III, Daims *et al.*, 1999; EURY498, Burggraf *et al.*, 1994; ALF1, Manz *et al.*, 1992; BET42a/Competitor, Manz *et al.*, 1992; GAM42a/Competitor, Manz *et al.*, 1992; DELTA495a, Loy *et al.*, 2002; SRB385, Amann *et al.*, 1992; SRB385Db, Amann *et al.*, 1992; HGC69a/Competitor, Roller *et al.*, 1994; LGC345mix, Meier *et al.*, 1999; Clost I, Küsel *et al.*, 1999] took place at 46°C for 4 h in standard hybridisation buffer containing formamide concentrations for the proper annealing of the probe with the target sequence (http:/www.microbial-ecology.de/probase/search.asp). The above mentioned probes were labelled with the fluorescent cyanine dyes Cy3 or Cy5 and were purchased from MWG Biotech (Ebersberg, Germany). The end concentration of each probe in hybridisation buffer was 10 ng/μL. After hybridisation the samples were washed at 48°C for 20 min in 100 mL washing buffer. Cells were counterstained with 4,6-diamidino-2-phenilindole (DAPI: 10 μg/mL). Two microlitres of DAPI was applied to each well and the slide was incubated for 15 min in the dark on ice. The excess DAPI was removed and rinsed carefully in $_{dd}H_2O$ and the slide was dried in the dark. Before microscopy, each well of the slide was embedded with Citifluor (Glycerol/PBS solution; antifading agent).

Microscopy, quantification of total cell numbers and FISH signals

Microscopy was performed using a CLSM 510 confocal laser scanning microscope coupled to an Axioplan2 epifluorescence microscope (both instruments from C. Zeiss, Jena, Germany). For detection of DAPI and used fluorescent dyes of probes, an enterprise laser: 351 nm and a helium neon (HeNe) laser 543 nm and 633 nm was used, respectively. A water immersion objective (63 × 1.4 A) was used for the visualisation of the hybridisation signals. For the quantitative analysis of the microbial population in our samples, the total cell number (TCN) per mL sample was calculated using DAPI as nucleic acid stain. For the evaluation of the FISH signals an area quantification method was applied. Twenty optical fields from each well of slight were obtained. The images were saved as TIFF-RGB file and the hybridised area was calculated as per cent against the area of cells stained with DAPI (% per area DAPI), with a CLSM image analysis programme.

Monitoring and analytical methods

Samples of raw and digested substrate for analyses were taken from the inflow, the hydrolytic and the methanogenic chamber. Analytical methods were based on German Standard Methods for the examination of water, wastewater and sludge (DEV, 1981). Monitoring of process parameters included: substrate quantities, quantity of biogas flow and digester temperature. Batch tests were performed to investigate the anaerobic biodegradability of the substrates used. The batch system had a volume of 19.3 L and was temperate by a water bath to a constant 42°C. Batch tests were performed according to DIN 38414 Part 8. Biogas produced in the batch tests was detected by a milli-gas-counter (Ritter®).

Results and discussion

Fluorescence *in situ* hybridisation

The first sampling was performed in the second month of operation with faecal sludge as the substrate. During this period the reactor was fed with low total solids load, less than 1%. The total microbial cell number was detected for the hydrolytic chamber to 1.36×10^9 ($\pm 1.23 \times 10^8$) TCN/mL and for the methanogenic chamber to 2.78×10^8 ($\pm 3.75 \times 10^7$) TCN/mL. The detection rate of *Bacteria* (detected with the probe EUB338mix) showed no significant differences between the hydrolytic and the methanogenic chamber and reached values of 20.8 and 20.6% of the total population. The slightly higher number of methanogenic *Archaea* (detected with the probe EURY498) in the hydrolytic chamber (19.2%) compared to the methanogenic chamber (15.7%) indicates a higher activity of methanogenic bacteria situated near to places where hydrolytic processes occur, i.e. mostly in the hydrolytic chamber of the reactor. Members of α-(ALFb1), β-(BET42a) and δ-(DELTA495a) *Proteobacteria* were detected in both chambers. No bacteria were detected belonging to the known hydrolytic groups of LGCmix (Gram-positive with low G + C) and Clostridium. Sulphate-reducing bacteria (SRB) were detected with SRB385 (Family *Desulfobacteriaceae*) on an average of 6.9 and 10.3% and SRB385Db (Family *Desulfovibrionaceae*) on an average of 1.3 and 2.6% in the hydrolytic and the methanogenic chamber, respectively. SRB can also grow in the absence of sulphate in syntrophic associations with methanogenic bacteria (Oude Elferink *et al.*, 1998). Hydrogen and acetate can serve as electron donors for the fermentative degradation of organic compounds for methanogens and sulphate-reducers, leading to a competition between these two microbial consortia.

The total microbial cell number after three months of operation time was detected for the hydrolytic chamber to be 9.15×10^8 ($\pm 9.8 \times 10^7$) TCN/mL and for the methanogenic chamber to be 7.64×10^8 ($\pm 4.96 \times 10^7$) TCN/mL. The detected signals of cells with FISH revealed a high microbial activity (Figure 1a and 1b). In detail, the population of Euryarcheota was 24.18% of the total population in the methanogenic chamber. The detection rate of *Bacteria* reached a value of 45.1 and 49.1% in the hydrolytic and methanogenic chamber, respectively. A high percentage of members of *Proteobacteria* was also detected. The detection average of sulphate-reducers was also significant, but was mainly observed in the hydrolytic chamber (SRB385: 20.4% and SRB385Db: 4.0%). Sulphate-reducers in the methanogenic chamber were detected to 7.7% (SRB385) and 4.1% (SRB385Db). Increased metabolic activity of the system was also observed as a result of a higher signal intensity of the probe signals obtained.

In the second operation period, the reactor was fed with a model substrate consisting of forage and cellulose, with a TS content of 7%. The new composition of the input material, with higher protein content (25% of TS) and with slow degradable cellulose, was made immediately remarkable by the microbial community. For the first time, members of Clostridium (detected with the probe Clost I) on an average of 4.7 and 3.2% of

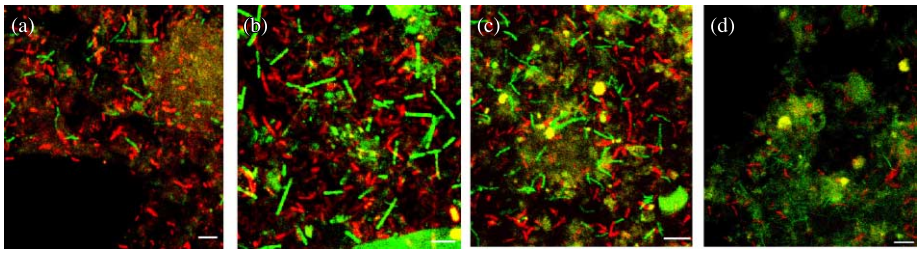

Figure 1 Confocal laser scanning microscopic images (single x–y sections) with hybridisation for *Bacteria* (EUB338mix, red cells) and for Euryarcheota (EURY498, green cells, mainly methanogens) from the hydrolytic (a) and methanogenic (b) chamber for the treatment of faecal sludge, and from the hydrolytic (c) and methanogenic (d) chamber for the treatment of the model substrate. Bar = 5 μm. Subscribers to the online version of *Water Science and Technology* can access the colour version of this figure from http://www.iwaponline.com/wst

the total population and Gram-positive bacteria (detected with the probe LGC345mix) on an average of 4.7 and 2.7% were detected in the hydrolytic and methanogenic chamber, respectively. To the phylogenetic group of Clostridia belong important proteolytic bacteria (Franks *et al.*, 1998) that are responsible for degradation of proteins and specifically for hydrolysis of slowly biodegradable substances such as cellulose. Sulphate-reducing bacteria SRB385 (1.4 and 15.5%) and SRB385Db (11.1 and 5.4%) were detected in both chambers. Although the total microbial cell number with DAPI showed a slight increase during this phase for the hydrolytic chamber with 4.55×10^9 ($\pm 2.81 \times 10^8$) TCN/mL and for the methanogenic chamber with 3.51×10^9 ($\pm 4.08 \times 10^8$) TCN/mL, the application of the FISH probes showed a lower microbial activity for the population of *Bacteria* and *Archaea.* This was confirmed by a lower signal intensity obtained by the specific probes (Figure 1c and 1d).

Modelling reactor performance and temperature influence on methanogenic activity

A professional software package consisting of Simba/Matlab/Simulink was used for the simulation study. The suggested biochemical parameter values given in Table 6.2 of the *Scientific and Technical Report* by Batstone *et al.* (2002) were set as initial values of the simulation model. However, it was not possible to simulate the two investigation periods, faecal sludge treatment and digestion of the model substrate, with one set of kinetic parameters. For simulation of faecal sludge treatment and the digestion of the model substrate the hydrolysis rate k_{hyd} was reduced from 10 to 1 d^{-1}. Furthermore, for the simulation of digestion of the model substrate the hydrogen inhibition constant for propionate utilisers $k_{I,H2,pro}$ was reduced from 10^{-5} to 7×10^{-6} kg-COD, the maximum uptake rate for acetate utilisers $k_{m.ac}$ was reduced from 16 to 14 kg-COD/(kg-COD·d) and the ammonia inhibition constant for acetate utilisers was reduced from 0.011 to 0.004 kmol-N/m^3. Simulation results are shown in Figure 2.

Figure 2 demonstrates that the calibrated model was capable of predicting biogas production for both investigation periods. Biogas produced in the first period was 10 times lower compared with the second period. This was due to the low total solids content of the faecal sludge used. By increasing the hydraulic load from 100 to 150 L/d at day 50, biogas production increased up to 125 L/d. A further increase of the hydraulic load to 200 L/d at day 105 led to a decrease in biogas production. Simulation results showed the same dependencies, but with a steeper increase and decrease in biogas production at the different hydraulic steps compared with the measurements. Digestion of the model substrate showed a drop in biogas yield from day 25 to day 40. This drop coincided with an increase in acetate and propionate concentrations in the effluent.

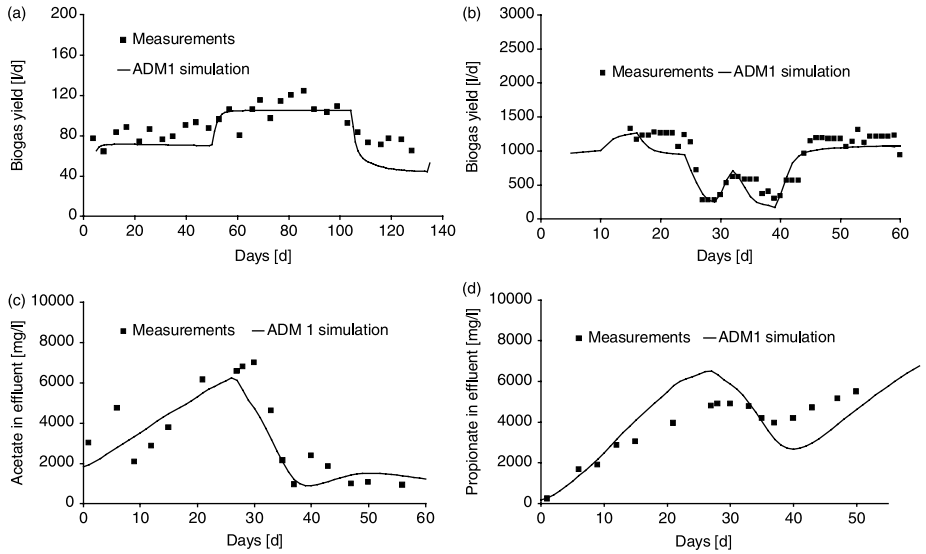

Figure 2 Comparison of measurements and ADM1 simulation results for (a) faecal sludge treatment and (b–d) digestion of the model substrate

Although acetate and propionate increased up to 7,000 mg/L the pH remained nearly constant. This can be explained by the buffer capacity of ammonia (here the sum of NH_4^+ and NH_3) which is released during the digestion of the model substrate containing a high amount of proteins. Ammonia nitrogen effluent concentrations reached 6,000 mg/L. Besides the positive effect of increasing the buffer capacity, free ammonia is inhibiting aceticlastic methanogenesis. Braun *et al.* (1981) suggested a free ammonia concentration of 0.15 g-N/L as the inhibitory level. The proportion of free ammonia depends on the pH and temperature and can be calculated according to following equation:

$$NH_3 - N = \frac{NH_4 - N}{e^{(6344)/(273+T)} \times 10^{-pH} + 1} \quad (1)$$

For an ammonia nitrogen concentration of 6,000 mg/L, a pH of 7.8 and a temperature of 55°C free ammonia nitrogen was calculated to 1.2 g-N/L. This is eight times higher than the inhibitory level suggested by Braun *et al.* (1981). Although anaerobic bacteria are often considered to have high adaptation capacity the drop in biogas yield together with the increasing VFA effluent concentrations can largely be explained by free ammonia inhibition. As a consequence, addition of the model substrate was stopped on day 30 for 7 days and the reactor content was diluted with wastewater to reduce total ammonia concentration. After the reduction of acetate below 1,000 mg/L the reactor was fed again with a reduced amount of forage. The overall system behaviour could be reproduced by the simulation model.

Batstone *et al.* (2002) suggested parameter values for ADM1, which are valid either for 35 or 55°C. A temperature of 45°C can be assumed as too high for mesophilic and too low for methanogenic organisms leading to a decreased methanogenic activity. As the temperature range between 45 and 55°C is investigated in this study, we enhanced substrate uptake rate of methanogenic organisms in ADM1 by a double Arrhenius equation of the following form:

$$k = b_1 \exp[a_1(T - 30)] - b_2 \exp[a_2(T - 30)] \quad (2)$$

where k is the relative methanogenic activity, and b and a are coefficients. Pavlostathis and Giraldo-Gomez (1991) demonstrated the applicability of equation 2 to model the

methanogenic activity of *M. arboriphilus*. The thermophilic anaerobic system investigated in this study was operated for several days at temperatures lower than 55°C, by which biogas productivity of the system at different temperatures was gained. Specific biogas production rate (in l/(kgVS·d)) observed at different temperatures was divided by the specific biogas production rate observed at 55°C. The values obtained can approximately be interpreted as the temperature-dependent relative methanogenic activity according to equation 2. Measured values are depicted in Figure 3. Based on the experimental results equation 2 was modified as follows:

$$k = 0.72 \exp[0.14(T - 48)] - 0.13 \exp[0.28(T - 48)] \quad (3)$$

The comparison between modelled and measured relative specific biogas production rate can be seen in Figure 3. Although only five measurements were available the temperature influence can be recognised and equation 3 was able to fit the measurements. The equation was therefore added to the substrate uptake rate of methanogens in ADM1 and was used for simulation of both investigation periods: faecal sludge treatment and digestion of the model substrate.

Modelling temperature influence on net energy production

Thermal energy which is consumed by digester operation can be mainly divided into energy required to compensate irradiation losses and energy required to heat the substrate to the operating temperature. These two energy forms can be calculated by the following equation:

$$E_C(t) = K_{\text{heat\_trans}} V_{\text{liq}} (T_{\text{liq}}(t) - T_{\text{ambient}}) \frac{2}{r} \frac{24}{1000} + Q_{\text{in}}\, c (T_{\text{liq}}(t) - T_{\text{substrate}}) \frac{1}{3.6} \quad (4)$$

where E_C is the energy consumed (kWh/d), $K_{\text{heat\_trans}}$ is the heat transfer coefficient (Wh/(m^2 h °C)), V_{liq} is the liquid volume (m^3), T_{liq} is the temperature of the substrate within the digester (°C), T_{ambient} is the ambient temperature (°C), r is the radius of the digester (m), Q_{in} is the reactor inflow (m^3/d), c is the heat capacity of the substrate (kJ/(kg °C)), $T_{\text{substrate}}$ is the temperature of the substrate fed (°C) and t is the temperature (°C) which was varied from 45 to 55°C. The term 2/r represents the surface to volume ratio of the cylindrical digester (m^2/m^3).

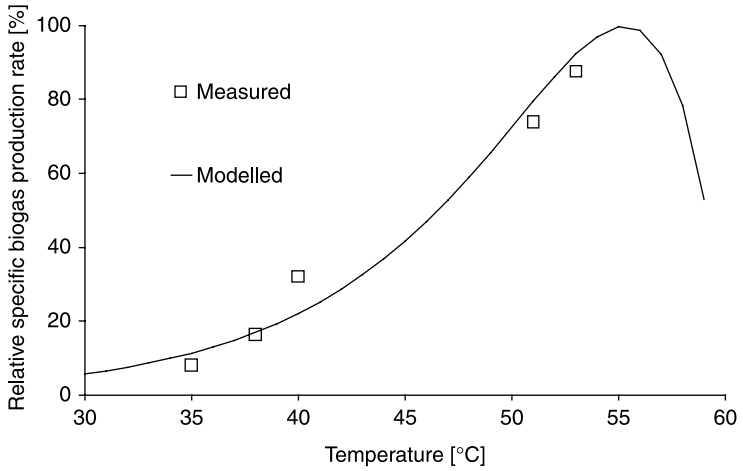

Figure 3 Modelled and measured relative specific biogas production rate

The production of biogas in a combined heat and power unit (CHP) produces thermal energy, which can be used to compensate the digester's loss of energy. We used the following equation to calculate energy production at different temperatures:

$$E_P(t) = Q_{\text{gas}}(t) P_{CH_4}(t) H_C \eta \tag{5}$$

where E_P is the energy produced (kWh/d), Q_{gas} the biogas produced (m^3/d), P_{CH_4} the methane content (%), H_C the calorific value of methane (9.94 kWh/Nm^3) and η the degree of efficiency (−).

Equations 4 and 5 together with the modified ADM1 enabled us to obtain the change of energy produced and consumed at different temperature ranges. The digester temperature was changed from 45 to 55°C and the ambient temperature was set constant to 5°C. The results are shown in Figure 4.

The energy produced was simulated by the modified ADM1. According to the model, and shown in Figure 4, the production of energy decreases when the temperature is lowered. The calculated values are given in per cent of the energy produced at 55°C. The energy consumed was calculated by equations 4 and 5. With decreasing temperature the energy requirements to compensate irradiation losses and to heat the substrate decline. This temperature-dependent saving of energy is shown in Figure 4 in per cent of the energy consumed at 55°C. The diagram shows that the reduction of temperature can have a positive effect on net energy production and that this effect depends on the substrate treated. For faecal sludge treatment, the percentage increase of energy saving is higher than the percentage decrease of energy due to a lower microbial activity only for temperatures lowered to 53°C. For the digestion of the model substrate, the same characteristics were calculated for temperatures lowered to a minimum of 46°C. Although the system showed instabilities when the model substrate was treated (Figure 2b–d) the percentage decrease in energy production was calculated to lower values compared with faecal sludge treatment. Moreover, the simulation model calculated a decrease of acetate in the effluent of 30% when the temperature was lowered to 50°C (not shown). This indicates a higher degree of stability at 50°C compared with 55°C. However, acetate increased again when the temperature was further decreased in the model. The maximum difference between energy saved and produced was calculated for 50°C indicating the optimal temperature in terms of net energy production. The fact that methanogenic activity was only slightly reduced in the model at temperatures down to 50°C can be explained by equation 1. Free ammonia is reduced from 1.2 g-N/L at 55°C to 0.9 g-N/l at 50°C. According to the simulation model, the release of free ammonia inhibition of methanogens at temperatures down to 50°C can compensate for a considerable part of the reduction of methanogenic activity

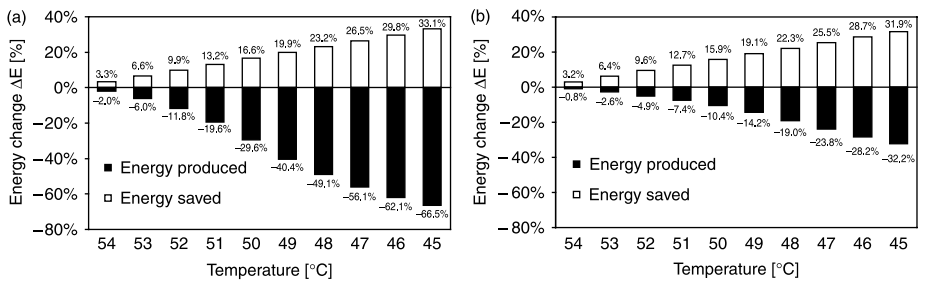

Figure 4 Modelled change of energy at different temperatures for (a) faecal sludge treatment and (b) digestion of the model substrate

due to the reduced temperature. At lower temperatures the reduction of methanogenic activity is predominant as shown in Figure 3 and calculated by equation 3.

Conclusions

- Fluorescence *in situ* hybridisation (FISH) was proved to be a useful technique to monitor microbial population during the whole investigation period. Process inhibition could be recognised by a lower signal intensity obtained by the application of the specific probes.
- Digestion of the protein-rich model substrate led to process instabilities due to free ammonia inhibition of aceticlastic methanogenesis.
- ADM1 was capable of simulating reactor performance for both investigation periods. The model was modified to allow the simulation of reactor performance at different temperatures.
- The simulation study indicated for the model substrate that reactor operation at 50°C leads to both the highest net energy production and better process stability. This can be explained by the release of free ammonia inhibition at reduced temperatures.

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