The effect of different mesophilic temperatures during anaerobic digestion of sludge on the overall performance of a WWTP in Sweden

J. Moestedt, J. Rönnberg and E. Nordell

ABSTRACT

This project was initiated to evaluate the effect of alternative process temperatures to 38 °C at the anaerobic digestion step in a Swedish wastewater treatment plant (WWTP) treating mixed sludge. The efficiency of the different temperatures was evaluated with respect to biogas production, volume of sludge produced and nutrient content in the reject water to find the optimum temperature for the WWTP as a whole. Three temperatures, 34 °C, 38 °C and 42 °C, were compared in laboratory scale. Increasing the process temperature to 42 °C resulted in process instability, reduced methane yield, accumulation of volatile fatty acids and higher treatment costs of the reject water. By decreasing the temperature to 34 °C, slightly higher sludge mass was observed and a lower gas production rate, while the specific methane produced remained unchanged compared to 38 °C but foaming was observed at several occasions. In summary 38 °C was proved to be the most favourable temperature for the anaerobic digestion process treating mixed sludge when the evaluation included effects such as foaming, sludge mass and quality of the reject water.

Key words | biogas, hyper-mesophilic, kinetics, sludge, temperature change, wastewater

INTRODUCTION

Anaerobic digestion is commonly used at wastewater treatment plants (WWTPs) to treat primary sludge from settling of the incoming wastewater, and secondary sludge which is derived from the biological treatment steps. At the WWTP in Linköping (Sweden), wastewater corresponding to 200,000 population equivalent can be treated annually and the process generates primary and secondary sludge that is treated by anaerobic digestion. The main purpose with the anaerobic digestion step is to stabilize the sludge and thus make it easier to dewater, as well as to reduce the sludge mass (Appels et al. 2008). During the digestion biogas is formed, which in Linköping is upgraded to vehicle fuel quality, 97% methane. Digestate from the digesters is dewatered from 3–4% total solids (TS) up to 25–30% of TS with screw presses (Ishigaki, Ishigaki Company Ltd, Japan). The reject water from the dewatering unit is recirculated back into the WWTP to reduce the ammonium nitrogen content in the reject water. Ammonium nitrogen in the reject water is typically an undesired additional nitrogen source and it is typically necessary to first be treated with dedicated cleaning steps such as a sequencing batch reactor, single reactor system for high activity ammonia removal over nitrite (SHARON®) or anaerobic ammonium oxidation. In Linköping the SHARON process is used, in which nitrification occurs via nitrite instead of nitrate due to active wash-out of nitrite oxidizers by selection of an appropriate hydraulic retention time (HRT) (Hellinga et al. 1998; Sri Shalini & Joseph 2012). The SHARON process is hence used and subsequent to this cleaning process, the reject water is recirculated to the incoming flow. The efficiency of the SHARON process is dependent on the incoming concentration of nutrients, i.e. ammonium nitrogen and phosphate. At present at Linköping WWTP, the reject water contains approximately 1,100 mg NH₄-N/L and 30 mg PO₄-P/L, resulting in a lack of phosphate and thus a need for dosage of phosphoric acid to the SHARON process for successful operation.

In general, either mesophilic (37–39 °C) or thermophilic (52–55 °C) temperatures are applied during anaerobic digestion. However, during the last couple of years the use of hyper-mesophilic temperatures (40–44 °C) has caught more attention for other types of substrates (Moestedt et al. 2014;...
Westerholm et al. 2015). Earlier studies indicated process instability during co-digestion processes in this temperature range (van Lier et al. 1993; Lindorfer et al. 2008a, 2008b). However, the hyper-mesophilic temperature has been applied with great results at Sweden’s largest co-digestion plant (Linköping biogas plant) for digestion of food and slaughterhouse wastes (Moestedt 2015). The higher temperature has been reported to select for efficient microorganisms responsible for syntrophic acetate oxidation as well as higher biogas production yields for digestion of thin stillage (Moestedt et al. 2014). At a WWTP the anaerobic digestion, however, is not an isolated process and the temperature should not be selected only based on biogas yield. Earlier studies have shown that an increased temperature might result in higher nitrogen mineralization, i.e. higher nutrient concentration in the digestate. Additionally, if a more efficient anaerobic digestion process is obtained, volatile solids (VS) reduction increases, which in turn affects the produced sludge mass. Moreover, lower process temperature might instead result in lower mineralization, thus reducing the load to the SHARON process.

This project was initiated to evaluate the effect of alternative process temperatures to 38 °C, which is applied presently at the Linköping WWTP. The efficiency of the different temperatures was evaluated with respect to biogas production, mass of sludge produced and nutrient content in the reject water to find the optimum temperature for the WWTP as a whole. Three temperatures, 34 °C, 38 °C and 42 °C, were compared in laboratory scale with substrate from Linköping WWTP.

MATERIAL AND METHODS

Experimental set-up

Three continuously stirred tank (CSTR) laboratory reactors with an active volume of 9 L (Nordell et al. 2011) were operated for in total 100 days. The reactors were agitated continuously at 80 rpm and fed semi-continuously (once a day) 7 days per week. Volume adjustment and sampling were performed 5 days per week, prior to daily feeding. Volumetric gas production was measured online with a Ritter milligas counter (MGC-10, Ritter, Germany) and methane concentration with a gas sensor (BlueSens, Germany). Specific gas production was calculated as normalized (1.01325 bar and temperature 273.2 K) amount of gas produced per amount of organic material (measured as VS) added to the reactor per day.

Inoculum was collected from the anaerobic full-scale process at Linköping’s WWTP (Tekniska verken i Linköping AB publ., Linköping, Sweden) and the inoculum was thus adapted to 38 °C. Fresh substrate (thickened mixed primary and secondary sludge) was collected each week to simulate similar conditions as in the full-scale process. The active volume of each reactor was initially completely filled with inoculum and the HRT was fixed and set to 20 days according to full-scale conditions while the organic loading rate (OLR) varied according to the variation of VS in the sludge. The VS was on average 5.1 ± 0.2% and the OLR was subsequently 2.49 ± 0.06 kg VS/(m3 d) (correlating to approximately 0.15 kg substrate/(kg VS inoculum d)). During a start-up period of one HRT (20 days) all parameters were kept constant according to full scale. At day 21 the temperature was changed, and was phased from 38 °C to 34 °C (reactor named 34 °C) and 42 °C (reactor named 42 °C) respectively for a period of 7 days. The third reactor was kept at 38 °C throughout the experiment.

Volatile fatty acid (VFA) content was analysed with a Clarus 550 gas chromatograph (Perkin Elmer, USA) with a packed Elite-FFAP column (Perkin Elmer, USA) for acidic compounds according to Jonsson & Borén (2002). Ammonium nitrogen (NH4-N) was analysed as the sum of NH4-N (aq) and NH3-N (aq) by distillation (Kjeltec 8200, FOSS in Scandinavia, Sweden) in an acidic solution (H3BO3) and NH4-N was then determined by titration with HCl (Titro 809, Metrohm, Switzerland). Kjeldahl nitrogen (kjel-N) was determined with the same procedure and equipment as NH4-N, with the exception that the samples were pre-treated with H2SO4 and subsequently heated to 410 °C for 1 h. The ammonia (NH3-N) concentration was calculated from the NH4-N concentration, pH and temperature according to Hansen et al. (1998). The pH was measured with a potentiometric pH meter at 25 °C using a Hamilton electrode (WTW Inolab, USA). Dry matter (DM) was measured by oven-drying at 105 °C for 20 h and VS was subsequently measured by combusting the DM sample at 550 °C for 3 h. Degree of degradation was calculated as VS-removal using TS and VS values in substrate and digestate respectively. Gas production rate for the methanogenesis (km) was measured according to Moestedt et al. (2015); the gas production rate was determined by fitting the daily gas production curve, which arises with semi-continuous feeding, to a lumped first-order kinetic equation. All error bars are calculated statistically as confidence interval using two-sided student’s T-test (α 0.05). For quantitative polymerase chain reaction (qPCR) analysis of the methanogenic groups, samples (50 mL) were withdrawn from the reactors and...
DNA was extracted in triplicate with aliquots from this sample according to Westerholm et al. (2012).

RESULTS AND DISCUSSION

The three reactors were performing equally during the start-up period with respect to biogas production, ammonium- and phosphate content etc. After the first HRT the temperature was gradually changed to 34 °C and 42 °C in two of the reactors during 1 week. After that, the processes were run for four additional HRTs, in order to ensure complete exchange of the ingoing/outgoing material and to reach steady state. Commonly, three HRTs are enough to reach steady state since by then 99% of the original reactor material has been replaced in a CSTR system. The fourth HRT was hence used to further evaluate the processes when steady state was present. Besides the material to be exchanged, the microbial community also needs to adapt to the new environment; within three HRTs only active microorganisms in the new environment originating from the original inoculation will be present. However, the time for adaptation of complex microbial communities may be difficult to predict and a further extension of the evaluation phase in this experiment after the first three HRTs may have been beneficial to determine the effects of different temperatures. During the first three HRTs after temperature change, no difference in specific methane production, methane content nor degree of degradation was observed, indicating that the change in temperature had minor effect on the VS-reduction and gas yield (Figure 1(a) and 1(b)). However, the profiles for ammonium (NH₄-N), ammonia (NH₃-N) and phosphate (PO₄-P) showed rapid differences for both the reactor with lower and higher temperature, respectively (Figure 1(c) and 1(d)).

Between day 30 and day 80 the NH₄-N was increased significantly for the reactor with 42 °C (1,670 mg NH₄-N/L) compared with 1,540 mg/L for the reactor running at 38 °C (Figure 2). The ammonium concentration also tended to be lower in the reactor applying 34 °C, but this change was not significant, compared with the 38 °C reactor. On the other hand, the difference in ammonium concentration between 34 °C and 42 °C was significant. In earlier experiments (unpublished), similar results with increasing NH₄-N concentrations with increasing temperature have been observed. This might indicate that the hydrolysis of proteins and amino acids is enhanced at elevated temperature' or that assimilation of ammonia into the cells had decreased following lower biomass at higher temperature. However, since neither the gas production nor degree of degradation was significantly affected during this period, the possible effect of enhanced mineralization of nitrogen-containing components did not result in further conversion of these components to biogas. The elevated temperature combined with higher NH₄-N resulted in increased NH₃-N as a consequence of the temperature and pH-dependent equilibrium between NH₄-N and NH₃-N (Gellert & Winter 1997; Hansen et al. 1998; Chen et al. 2014). Hence, the NH₃-N reached 160 mg/L in 42 °C compared with 90 mg/L in 38 °C and 50 mg/L in 54 °C. The ammonia level at 42 °C was below most reported levels for inhibition but is close to levels known to cause a shift in the methanogenic pathway from acetotrophic to hydrogenotrophic methanogenesis together with syntrophic acetate oxidation, a shift that might cause process instability (Rajagopal et al. 2013; Moestedt et al. 2016; Westerholm et al. 2016). The changes in phosphorous concentration were only significant when comparing 34 °C with 42 °C (Figure 2). The lower NH₄-N and PO₄-P in the 34 °C reactor could be due to lower microbial activity at lower temperature, however not sufficiently lower to be detected in gas production. It should be considered that the change in ammonia and possibly also phosphate could cause a change in the microbial community as described above, which in turn could be correlated to changes in process performance.

The fact that the later steps in the anaerobic digestion, i.e. oxidation of fatty acids and/or methanogenesis, were not positively affected at elevated temperature and the lower microbial activity at lower temperature was also confirmed when studying the gas production rate constant for methanogenesis (Figure 1(e)). The gas production rate constant kₘ measures how fast the methane gas potential is reached each day and not the total gas production. Two different processes might have the same total gas yield but different gas production rates; a lower rate indicates that the process needs more time to reach the same total gas yield and hence is more stressed (Moestedt et al. 2015). When comparing kₘ for the 38 °C reactor with the 34 °C it is clear the gas production rate, and thus microbial activity, decreased directly after changing temperature (from day 20 and onward). Moreover, in the reactor running at 42 °C, the kₘ started to decrease already at day 20 indicating a stressed process. When the rate continued to decrease until day 80 by >90% compared to the control reactor the reduced rate also resulted in VFA accumulation that could be detected in this reactor, which was not detected in the other two reactors at any time (Figure 1(f)). The VFA accumulation correlated with a small decrease in methane
content in the reactor, which further indicated that the methanogenic activity could no longer match the initial steps of the anaerobic digestion (Figure 1(f)). VFA accumulation when digesting sludge from a WWTP, applying the classic 38 °C temperature, is uncommon due to the difficulty in reaching a high hydrolysis rate, which is why the VFA-oxidizing steps (acetogenesis and methanogenesis) almost never are the bottle neck in the anaerobic process (Appels et al. 2008). Therefore the combined results of $k_m$ and VFA clearly indicate that the methanogenic step was negatively affected by the increased temperature. The cause of the lower $k_m$ at higher temperature could be an effect of the rapid increase of NH$_3$-N rather than the absolute concentration that was lower than concentrations reported for inhibition. The increase in NH$_3$-N was 80% within the first two HRTs due to the higher process temperature. Hence the decrease of $k_m$ correlates with the fact that the microbial community was exposed to the higher NH$_3$-N, resulting in a microbial selection towards more ammonia-tolerant microorganisms. As a result of increasing VFA and

![Figure 1](https://iwaponline.com/wst/article-pdf/76/12/3213/241882/wst076123213.pdf)
lower gas production rate, the specific gas production subsequently decreased at 42 °C after 80 days. The effect of elevated temperature to a hyper-mesophilic range (42–44 °C) has in other studies resulted in increased methane yield and km when treating food waste (Moestedt et al. 2017) but other results have been observed with other substrates (Westerholm et al. 2015). From the DNA analysis it was furthermore observed that the methanogenic population was affected by the increase but not the decrease in temperature (Figure 3). The DNA analysis of the methanogenic groups indicated that Methanosetaeaceae was not detected, neither was any targeted syntrophic acetate-oxidizing bacteria (SAOB) except for Clostridium ultunense, which was constant throughout the experiment in all reactors. The relative abundance of the different groups was equal at both the starting point (day 16) and at the end of the experiment (day 100) for 34 °C and 38 °C, indicating no effect on the composition of the methanogenic population by decreased temperature. At 42 °C on the other hand, the abundance of the otherwise dominant hydrogenotrophic Methanomicrobiales was replaced by an increase of hydrogenotrophic Methanobacteriales and to a greater extent an increase of the mixotrophic Methanosarcinaceae. In an earlier study, with similar change of temperature to 42 °C the Methanomicrobiales group instead proliferated together with Tepidanaerobacter acetatoxidans and the gas production also increased (Moestedt et al. 2014). One possible explanation for the difference in microbial response and gas production might be that this study includes rather low ammonia concentration in comparison. High ammonia is known to select for the syntrophic acetate-oxidizing pathway with hydrogenotrophic methanogens such as Methanomicrobiales (Schnürer & Nordberg 2008). Methanosarcinaceae is, however, known to be both hydrogenotrophic and acetotrophic and relatively resistant to changes of NH₃-N concentration and temperature (De Vrieze et al. 2012). In a screening of Swedish biogas plants, all WWTPs were dominated by acetotrophic rather than hydrogenotrophic methanogens, indicating that SAOB and hydrogenotrophic methanogenesis play a minor role when treating such substrates, which might explain the absence of positive effects at 42 °C (Sundberg et al. 2013). Hence the rather quick increase in NH₃-N to levels in between the optimum for both acetotrophic and SAO/hydrogenotrophic methanogenesis could result in a situation where neither community proliferates and thereby a reduced microbial activity is obtained (low kₘ) and VFA accumulation.

Foaming and effects on other processes at the WWTP

This experiment was performed during winter time with fresh primary and secondary sludge each day. It is well known that problems with foaming due to filamentous bacteria such as Microthrix parvicella in secondary sludge increase with lower temperature, for example during the winter season. Foaming in anaerobic digestion has been reported to increase with lower temperature and hence this was an important parameter to evaluate during this experiment. Foaming was not quantitatively measured during this experiment, but a thick layer of foam was observed in the 34 °C reactor throughout the experiment, and a thinner layer was also observed in the 38 °C reactor. Over-foaming occurred at four occasions at 34 °C while there was no indication of foaming whatsoever at the surface at 42 °C. This was expected since the filamentous bacterium Microthrix parvicella has an optimum
temperature <35 °C and, hence, the use of 34 °C in anaerobic digestion processes at a WWTP may increase the risk of foaming compared to higher temperatures (Rossetti et al. 2005; Ganidi et al. 2009). Foaming was not treated during the experiment; if necessary tubing and measuring equipment was cleaned to remove foam.

The change of temperature affects the conditions for subsequent steps of the WWTP and the total economy. Factors affecting the economy of the WWTP are energy for heating of the digesters; electricity need for aeration and requirement for extra organic substrate to the SHARON correlating to the NH₄-N concentration of the reject water; need for phosphoric acid dosing correlating to the PO₄-P concentration of the reject water; gas production and finally the drying and disposal of dewatered sludge according to the TS content of the digestate. The economic evaluation, excluding gas revenues (illustrated in Figure 4), shows that the heating is the largest factor determining the economy of the process changes. The TS content at 34 °C was slightly higher (2.6% relative amount) compared to 38 °C, however not a significant change, while 42 °C was marginally higher but on average had the same TS as 38 °C. Hence this would cause an increase of sludge treatment for both alternatives (Figure 4).

The increased phosphate ration observed in the 42 °C would be positive for the SHARON since a higher phosphate concentration may decrease the need for phosphoric acid. In fact, 42 °C would limit the phosphoric acid addition to almost zero; however, the increased absolute ammonium still results in higher costs following increased aeration and dosing of organic substrate (e.g. ethanol) to the process (Figure 4). In summary, the economic evaluation shows that a decrease in temperature would be economically favourable (however, to a limited amount on a yearly basis) when assuming equal gas production as 38 °C despite higher treatment costs for the sludge. However, the risk of foaming has not been included, which is a strong incentive to keep the temperature above 35 °C. Since increased temperature leads to higher ammonia and heating costs, the reduction in TS and gas production would have needed to increase significantly to ensure a positive economic result; however, this was not the case (Figure 4).

CONCLUSIONS

This study investigated the effect of changing the temperature at the anaerobic digestion step at Linköping WWTP in laboratory scale from 38 °C to hyper-mesophilic (42 °C) and sub-mesophilic (34 °C). Although the evaluation only proceeded for four HRTs after temperature change, increasing the process temperature to 42 °C caused process instability, reduced gas yields, accumulation of VFA and higher treatment costs of the reject water. By decreasing the temperature to 34 °C, slightly higher sludge mass was indicated and a lower gas production rate, while the specific methane produced remained unchanged compared to 38 °C but foaming was observed on several occasions. In summary, the now-applied temperature (38 °C) was the most favourable temperature in this experiment, when evaluating the total effects on the WWTP and avoiding foaming.
REFERENCES


First received 12 January 2017; accepted in revised form 5 June 2017. Available online 7 September 2017