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Interaction between temperature and ammonia in mesophilic digesters for animal waste treatment

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ABSTRACT

Four anaerobic sequencing batch reactors (ASBRs) were operated during a period of 988 days to evaluate the effect of temperature, ammonia, and their interconnectivity on the methane yield of anaerobic processes for animal waste treatment. During period 1 (day 0–378), the methane yield was 0.31 L CH_4/g volatile solids (VS) for all digesters (with no statistical differences among them) at a temperature and total ammonium-N levels of 25 °C and ~1200 mg NH_4^+-N/L, respectively. During period 2 (day 379–745), the methane yield at 25 °C decreased by 45% when total ammonium-N and ammonia-N were increased in two of the four ASBRs to levels >4000 mg NH_4^+-N/L and >80 mg NH_3-N/L, respectively. During period 3 (day 746–988), this relative inhibition was reduced from 45% to 13% compared to the low-ammonia control reactors when the operating temperature was increased from 25 °C to 35 °C (while the free ammonia levels increased from ~100 to ~250 mg NH_3-N/L). The 10 °C increase in temperature doubled the rate constant for methanogenesis, which overwhelmed the elevated toxicity effects caused by the increasing concentration of free ammonia. Thus, the farmer/operator may alleviate ammonia toxicity by increasing the operating temperature within the mesophilic range. We extrapolated our data to correlate temperature, ammonia, and methane yield and to hypothesize that the difference between high- and low-ammonia reactors is negligible at the optimum mesophilic temperature of 38 °C.

1. Introduction

Because of increased costs for nonrenewable energy sources, application of anaerobic digestion (AD) as an integrated (or even a core) part of a variety of waste treatment systems has reemerged as an economical alternative (Agler et al., 2008; Bocher et al., 2008; Foresti et al., 2006; Lettinga, 2005; Zhang and Zhang, 2002). These systems include farm-based operations, industrial and agricultural processing, centralized and decentralized wastewater treatment plants, and solid waste recovery facilities. Animal waste digestion plays an important role in a farm-based system approach because of several reasons: the high energy density of animal waste; the vast quantities of animal waste produced annually (1.4 billion tons of waste in the US) (USDA, 2006a, b); the use of concentrated animal feeding operation (CAFO), resulting in a high animal population in a relatively small land area (Sapkota et al., 2007); and the large quantities of nutrients that must be liberated for subsequent recycling on the farm, all of which make treatment necessary.

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Implementation of AD on farms is not without problems, and a high failure rate has been reported (Lusk, 1998). Such high failure rates are believed to be due to poor design, construction, and/or operation, and inadequate acclimation of biomass to, for example, high-ammonia levels (Angenent et al., 2002). High concentrations of total ammonium (dissociated ammonia [i.e., free NH_3] plus ammonium [NH_4^+]) in animal waste digesters are common because ammonia is an end product during the breakdown of urea- and protein-rich animal waste (Angelidaki et al., 2003). Many studies have demonstrated the inhibitory effects of free ammonia to methanogens (Angelidaki and Ahring, 1993; Kadam and Boone, 1996; Koster and Koomen, 1988; Lu et al., 2008; Robbins et al., 1989; Sprott and Patel, 1986; Steinhaus et al., 2007; Tezel et al., 2006), leading to a suppression of methane formation during AD. These results are often conflicting each other with regards to the inhibitory free ammonia threshold concentration, which can be explained by different environmental conditions, the complexity of anaerobic digestion systems, or by overestimations of the free ammonia concentrations (Hafner et al., 2006). In addition, these reports show only a correlation between ammonia levels and one other parameter, such as biogas production, acids accumulation, or methanogenic growth and do not include the interaction with other variables, such as temperature and pH (these parameters are typically fixed). The correlation between only two parameters may not be a good approach to infer information from the complex system of anaerobic digestion for which environmental conditions vary over the operating period (such as operating temperatures) and where the ammonia/ammonium species serves as a high capacity buffer. Animal waste digesters, therefore, maintain their pH at relatively high levels within the desirable range for biological processes even during periods of acid accumulation (Angenent et al., 2002).

The operating temperature of digesters is crucial for the stability of anaerobic systems, and may vary considerably throughout the astronomical year, especially for nonheated lagoon-type digesters. Temperature plays a major role in both thermodynamics and kinetics of the reactions mediated by microbes. For example, methanogenic activity increases as the temperature increases within the mesophilic range (Masse and Masse, 2001). In addition, chemical equilibriums are also affected by the operating temperature, especially for the concentration of free ammonia at a fixed total ammonium concentration. The ratio of free ammonia to the total ammonium will be much higher at higher temperatures. Since free ammonia is inhibiting to methanogenesis, higher temperatures can inhibit methane generation in anaerobic digesters. For this reason, ammonium-, urea-, and protein-rich wastewaters are difficult to treat under thermophilic conditions (i.e., 55–65 °C) even though the kinetics are favorable compared to mesophilic conditions (i.e., 25–37 °C) (Angelidaki and Ahring, 1994; El-Mashad et al., 2004; Bocher et al., 2008; Harris and Dague, 1993; Mackie and Bryant, 1995).

Here, we investigated the performance (in terms of methane yields and volatile fatty acids [VFAs] accumulation) of anaerobic digesters treating swine waste, focusing on the evaluation of two *interdependent* variables: operating temperature and free ammonia concentrations. For farm-based anaerobic digestion in temperate climates, thermophilic conditions are not feasible due to the energetic costs of heating large volumes of animal waste. We, therefore, studied the long-term effect of total ammonium concentrations on methane generation under mesophilic conditions only. By studying the long-term effect of different ammonia concentrations on the methanogenic activity of the biomass in our digesters at two different temperatures (25 °C and 35 °C), we found an interaction among temperature, ammonia, and methane yield for controlling digester imbalance and upsets.

2. Material and methods

2.1. Reactor setup

The ASBR experimental setup was according to Angenent et al. (2008) (Fig. S1), and consisted of: (a) four 5-L ASBRs made of glass with a cylindrical shape (S.1); (b) four independent biogas collection systems, each consisting of a 2-L bottle to collect and to distribute potential foam, a gas bag to prevent a pressure drop in the headspace during effluent discharge, a gas sampling port, and wet-test gas meter from Schlumberger Industries, Dordrecht, The Netherlands (Model 1L); (c) Masterflex pumps (Cole Parmer Instrument, Chicago, IL, USA) to pump digester effluent and to recirculate biogas; (d) Programmable timers (Chron Trol corporation, San Diego, CA, USA) to operate the four independent biogas recirculation pumps for intermittent mixing, which consisted of 1 min every hour at 1.6 L/min; and finally; (e) two heaters (Polyscience heating recirculator, model 210, Niles, IL, USA) to maintain a constant temperature of 25 ± 0.5 °C or 35 ± 0.5 °C.

2.2. Reactor start-up and feeding

All four of the reactors (R1, R2, R3, and R4) were inoculated with anaerobic granular sludge from an expanded granular sludge bed (EGSB)-biobed digester that treats preacidified brewery wastewater from Anheuser-Busch Company, St. Louis (MO). This sludge was blended to destroy the granular characteristics prior to inoculation. Immediately after inoculation, the ASBRs were flushed with nitrogen and methane gases for 15 min to create anaerobic conditions. Swine waste (i.e., faeces, urine, wasted swine food, flush water) was obtained from Premium Standard Farms, Kirksville (MO), diluted with Saint Louis (MO) tap water to a concentration of 20 g volatile solids (VS)/L, stored at −20 °C, and was thawed just before feeding. Five different batches of swine waste (F1–5) were fed throughout the operational period of ~1000 days with new batches starting on days 1, 78, 310, 451, and 557. Swine waste particles were smaller than 2.4 mm due to the use of a screen on the farm. The four ASBRs were fed every other day, which resulted in a cycle of 48 h with an instantaneous feed period, a ~47-h react period, a 1-h settling period, and a 2-min decant period.

2.3. Physical and chemical analyses

Total solids (TS), VS, soluble chemical oxygen demand (SCOD), total volatile fatty acids (VFAs), and sludge volume index (SVI) were performed according to *Standard Methods* (APHA, 1998). Total ammonium-N (ammonia-N plus ammonium-N) was measured using an ammonia electrode (Model 95-12, Thermo Electron Corporation, Beverly, MA). The pH in the digester

effluent was determined using a pH electrode (Model Oakton 510, Fisher Scientific, Vernon Hills, IL). In addition, we measured individual VFAs by gas chromatography (Varian GC 3400, Varian Associates, Inc., Sunnyvale, CA) with a flame ionization detector. The temperatures of the injector and detector were 230 °C, while the column temperature was increased from 100 °C to 199 °C over a 15-min period. We used an SPB™-5 fused silica capillary column with dimensions of 30 m × 0.53 mm × 1 μm (Supelco, Bellefonte, PA). Helium and hydrogen were used as carrier and ignition gases at 30 and 1 mL/min, respectively. Methane content of the biogas was determined by gas chromatography (Series 350, Gow-Mac Instruments Co., Lehigh Valley, PA) with a thermal conductivity detector. The temperatures of the injector, detector, and column were 50 °C, 115 °C, and 25 °C, respectively. The column used was a 1.22 m × 3.18 mm o.d. 20% DC-200 Chromosorb P (Varian, Inc., Palo Alto, CA), and helium gas was utilized as the carrier gas at a flow rate of 60 mL/min.

2.4. *Methanogenic activity measurements and free ammonia calculations*

The methanogenic activity test was adapted from a protocol described by Rinzema et al. (1988). Briefly, biomass samples were taken from the ASBRs and added into 250-mL serum bottles along with a macro- and micro-nutrient and buffer solution with acetate as the sole carbon source (Rinzema et al., 1988). The pH was initially maintained at 7.6, the temperature was maintained at 25 °C or 35 °C, and the total ammonium-N concentration was adjusted accordingly. The acetate-utilization rates for biomass from the high-ammonia digesters (R4) were measured with serum bottles similar to the methanogenic activity test at a high free ammonia concentration close to R4. We performed this test with biomass sampled on days 626 and 809 at temperatures of 25 °C and 35 °C, respectively. Acetate was used as single carbon source at an initial concentration of ~3000 mg/L, and measured with GC once every day for a week. The acetate-utilization rate was then calculated through a first-order kinetic model. The free ammonia-N concentration was calculated from the measured total ammonium-N concentration according to:

$$[\text{Total} - \text{N}] = [\text{NH}_3 - \text{N}] + [\text{NH}_4^+ - \text{N}] \quad (1)$$

$$[K_a] = \frac{[\text{NH}_3 - \text{N}][\text{H}^+]}{[\text{NH}_4^+ - \text{N}]} \quad (2)$$

$$\text{NH}_3 - \text{N} = \frac{[\text{Total} - \text{N}]}{\left[1 + \frac{10^{-\text{pH}}}{10^{-\text{pK}_a}}\right]} \quad (3)$$

where: K_a (ammonium/ammonia) is $10^{-9.25}$ and $10^{-8.95}$ at 25 °C and 35 °C, respectively. We extrapolated our data using Mathematica 6.0 (Wolfram Research, Inc., Champaign, IL) to estimate the interactions between the methane yield, the temperature, and the total ammonium-N.

2.5. *Experimental design*

During the first operating period (period 1) from day 1 to 378, the solids loading rate (SLR) was periodically increased from its start-up value of 1.0 to 2.1, 3.1, and 4.0 g VS/L/day, while keeping a relatively low total ammonium-N level of ~1200 mg N/L at 25 °C. This resulted in decreasing hydraulic residence times (HRT) of 20, 10, 6.67, and 5 days, respectively. Here, we increased the VS loading rate whenever the daily biogas production rates were within 5% of their average values allowing a minimum time period of one hydraulic residence time (HRT). We assume pseudo steady-state biogas production rates for these operating periods (Bocher et al., 2008). During the second operating period (period 2) from day 379 to 745, an elevated total ammonium-N concentration of ~4000 mg N/L was applied to two ASBRs (R3 and R4) at 25 °C by adding NH_4Cl (Table 1) and NH_4OH (to maintain a similar pH in all reactors), while the other two ASBRs (R1 and R2) remained at the total ammonium-N concentration of ~1200 mg/L at 25 °C. The design SLR of 4 g VS/L/day was utilized from day 280 to 557 after which a new batch of feed (F5; containing a higher concentration of acetic acid compared to F1–4) forced us to lower the SLR to circumvent instability. The SLR was decreased to 2.2 g VS/L/day at day 584, and was maintained at this level throughout the remaining operating period. During the third operating period (period 3) from day 746 until the end of the operating period, the temperature of all four digesters was increased to 35 °C, while total ammonium-N levels remained similar compared to period 2. At the end of the operating period for R3 and R4, we increased the total ammonium-N level to ~5200 mg N/L (Table 1). Throughout the operating period, the pH levels in R1–4 were maintained between ~7.5 and 7.6 (except during unstable periods). The biogas production rate data were corrected for standard temperature and pressure.

3. Results

3.1. *Period 1*

Methane yields, which were corrected for standard temperature and pressure, were similar among ASBRs (analysis of variance [ANOVA]: $n = 16$; $P > 0.99$; $F = 0.004$; $\propto = 0.05$) during period 1 when biogas production rates increased with increasing VS loading rates (Fig. 1A). We had anticipated this result because the same swine waste was fed to each of the reactors while the environmental conditions, such as total ammonium-N and free ammonia-N concentrations, were similar (Fig. 1B and C). Therefore, we report the pooled methane yield of 0.31 L CH_4/g VS fed ($n = 16$; $R^2 = 0.91$, standard error [SE] = 0.09) based on the amount of VS fed to all four digesters at four different SLRs (1–4 g VS/L/day) (Fig. 2). Similar to the methane yield, methane content and other chemical and physical analyses did not show statistical differences among reactors during periods of pseudo steady-state gas production rates. The mean values for methane content among all digesters were 76% ± 0.02%, 71% ± 0.05%, 68% ± 0.04%, and 68% ± 0.03% at SLRs of 1.0, 2.1, 3.1, and 4.0 g VS/L/day, respectively. In addition, the total and individual VFA concentrations were similar among the four reactors (<1000 mg CH_3COOH/L) at the 4.0 g VS/L/day loading rate (Fig. 1D and Fig. 3). The VS removal efficiencies were 64% ± 1%, 61% ± 4%, 53% ± 3%, and 51% ± 3% for all reactors at SLRs of 1.0, 2.1, 3.1, and 4.0 g VS/L/day, respectively. A sharp drop in the biogas production rate was observed on day 310 for all ASBRs (Fig. 1A) due to a change in feed batch (Table 2), indicating a strong effect of animal waste composition on reactor performance despite a similar VS concentration of ~20 g VS/L.

Table 1 – Experimental design with average total ammonium concentrations.

Digesters	Parameters	Period 1 (days)			
		0–190	191–234	235–280	281–378
R1, R2, R3, and R4	Temperature (°C)	25	25	25	25
	Total ammonium-N (mg/L)	~1000	~1000	~1000	~1200
	SLR (g VS/L/day)	1.0	2.1	3.1	4.0
			Period 2 (days)		
			379–583	584–745	
R1 and R2	Temperature (°C)		25	25	
	Total ammonium-N (mg/L)		~1600	~1600	
	SLR (g VS/L/day)		4.0	2.2	
R3 and R4	Temperature (°C)		25	25	
	Total ammonium-N (mg/L)		~4400	~4400	
	SLR (g VS/L/day)		4.0	2.2	
			Period 3 (days)		
		746–835	*836–938*	*939–966*	*967–988*
R1 and R2	Temperature (°C)	35	35	35	35
	Total ammonium-N (mg/L)	~1800	~1800	~1800	~1800
	SLR (g VS/L/day)	2.2	2.2	2.2	2.2
R3 and R4	Temperature (°C)	35	35	35	35
	Total ammonium-N (mg/L)	~4400	~4900	~5200	~5200
	SLR (g VS/L/day)	2.2	2.2	2.2	2.2

When ammonium salts were artificially added to the reactors, the concentrations are given in an italic font.

3.2. *Period 2*

The total ammonium-N concentration was increased from 1200 to 4000 mg N/L (in R3 and R4 on day 378) after a pseudo steady-state biogas production rate was achieved (Fig. 1A and B). With methanogenic activity tests of samples from period 1, we had found a ~40% inhibition of the methanogenic activity with biomass from R2 and R4 at the total ammonium concentration of 4000 mg N/L when compared to 1200 mg N/L at a pH value of 7.7 ± 1.0 and a temperature of 25 °C (Fig. 4). Such a relative inhibition was thought to be suitable for a bioreactor study in which the long-term effects of and acclimation to free ammonia needed to be studied without completely inhibiting methanogenesis (i.e., not exceeding the ammonium threshold level). At the end of the methanogenic activity test, the pH in the serum bottles had risen to 7.8, which resulted in a higher concentration of free ammonia for the MAT analysis compared to the ASBRs that were operated at a pH level of 7.6 (137.1 and 87.6 mg NH_3-N/L, respectively). Similarly to the methanogenic activity data that were taken over a two-day period, R3 and R4 showed a ~40% reduction in biogas production rates compared to R1 and R2 throughout the operating period 2 of over 300 days (Fig. 1A). This period included an operating upset after feeding the more potent F5 on day 557 (Table 2, Fig. 1A, D, and Fig. 3).

After reducing the SLR to 2.2 g VS/L/day (Table 1), the methane yields for the period of pseudo steady-state biogas production rate at the end of period 2 were 0.34 ± 0.02, 0.29 ± 0.02, 0.18 ± 0.02 and 0.17 ± 0.02 L CH_4/g VS fed for R1, R2, R3, and R4, respectively. The increase in ammonia resulted in a 45% lower methane yield for the arithmetic mean of R3 and R4 (0.17 L CH_4/g VS fed – statistically not different: [ANOVA: $n = 60$; $P = 0.10$; $F = 2.77$; $\propto = 0.05$]) compared to the mean of R1 and R2 (0.31 L CH_4/g VS fed – statistically different: [ANOVA: $n = 60$; $P < 1 \times 10^{-3}$; $F = 28.9$; $\propto = 0.05$]). We also performed methanogenic activity tests with samples taken from all digesters at the end of period 2 on day 558. The temperature and pH levels for the serum bottles were 25 °C and 7.5 ± 0.1, respectively. The mixed liquor (biomass) from R1 and R2 showed a methanogenic activity of 0.29 ± 0.04 and 0.30 ± 0.06 g COD–CH_4/g volatile suspended solids (VSS)/L at a total ammonium concentration of 1140 and 1000 mg N/L, respectively, while biomass from R3 and R4 showed an activity of 0.08 ± 0.02 and 0.07 ± 0.01 g COD–CH_4/g VSS/L at a total ammonium concentration of 4300 and 3700 mg N/L, respectively. The methanogenic activity for acclimated biomass from R3 and R4 at a total ammonium concentration of ~4200 mg N/L was lower than for nonacclimated biomass from R2 and R4 at this concentration at the end of period 1 due to accumulation of nondegraded VSS during period 2 (Fig. 4). From these bioreactor and methanogenic activity data, we did not find an obvious acclimation of the methanogenic biomass to high levels of free ammonia over an operating period of more than 300 days.

3.3. *Period 3*

Increasing the operating temperature from 25 °C to 35 °C, increased the biogas production rates in all four ASBRs. In addition, the difference in methane yields between the low-ammonium reactors (R1 and R2) and the high-ammonium ASBRs (R3 and R4) was decreased (Fig. 1A). This difference in methane yield was 13% for the mean of R1 and R2 (0.36 L CH_4/g VS fed) and the mean of R3 and R4 (0.31 L CH_4/g VS fed), while this had been 45% at the end of period 2. The individual methane yields during period 3 were 0.36 ± 0.02, 0.36 ± 0.02,

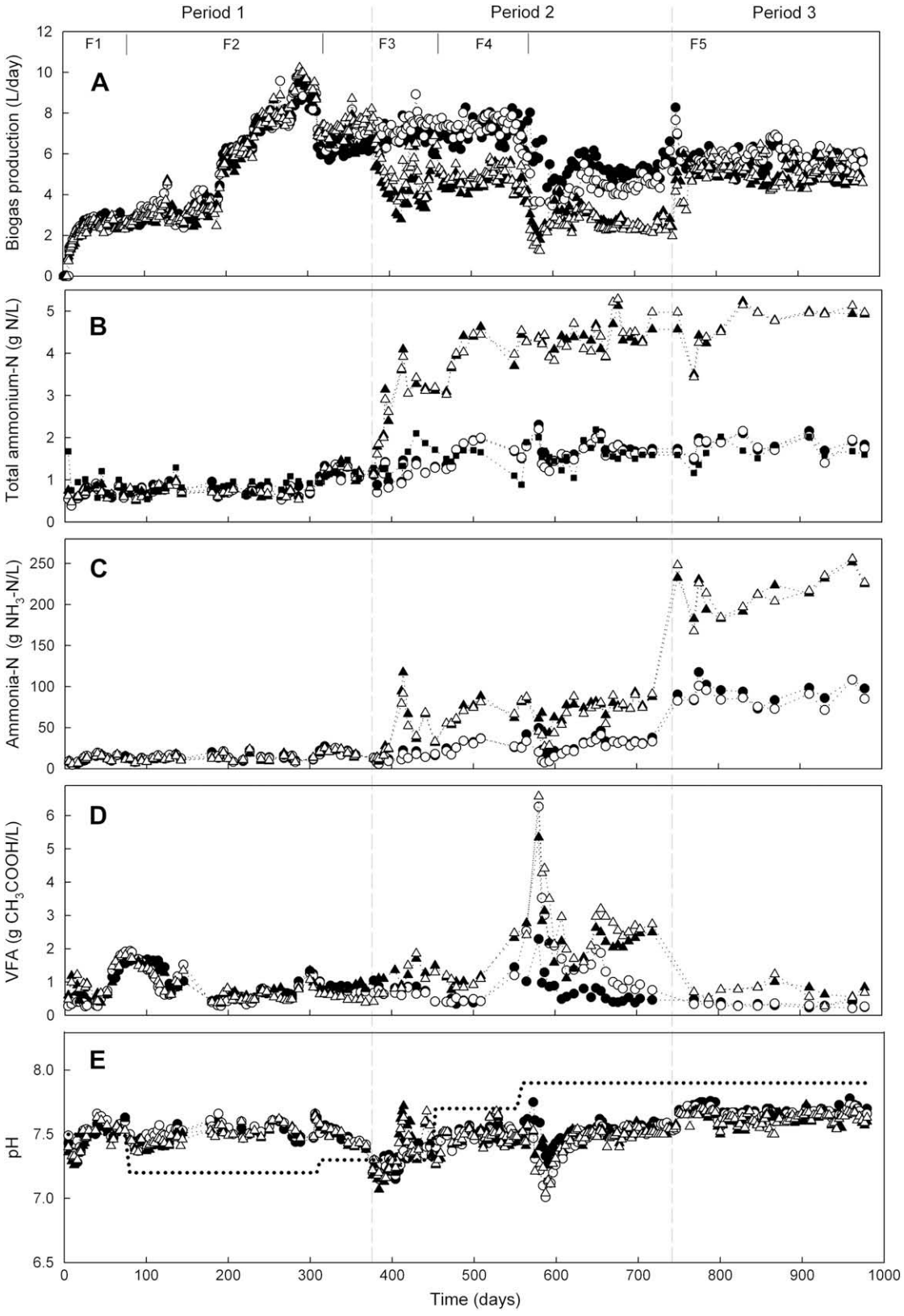

Fig. 1 – Performance data: (A) biogas production rate corrected for standard pressure and temperature; (B) total ammonium-N concentration (ammonium-N concentration plus ammonia-N concentration); (C) free ammonia-N concentration; (D) total volatile fatty acid concentration; and (E) pH levels (dotted line represents pH level of influent). Symbols represent reactors R1 (●), R2 (○), R3 (▲), and R4 (△) for panels A–E, and influent (■) for the total ammonium concentration (panel B).

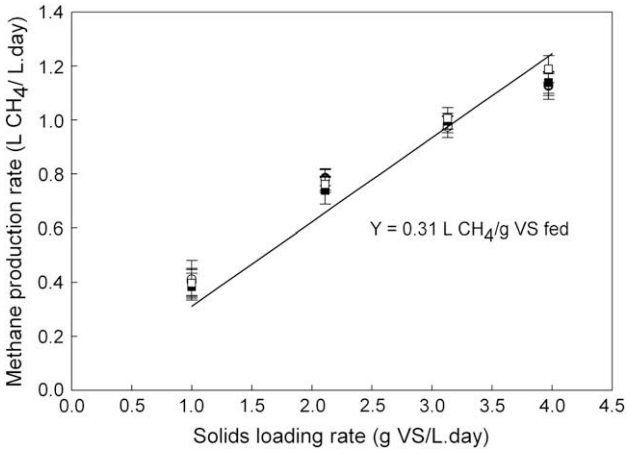

Fig. 2 – Methane production rates corrected for standard pressure and temperature over the VS loading rate for F1 and F2. The methane yield for the VS fed was obtained by linear regression analysis on data that were pooled from all digesters during each steady-state period for period 1 (methane production rates for F3 were not included).

0.31 ± 0.03 and 0.32 ± 0.03 L CH_4/g VS fed for R1, R2, R3, and R4, respectively. The methane yields for the low- and high-ammonia ASBRs were significantly different (ANOVA: $n = 248$; $P < 0.01$; $F = 59.0$; $\propto = 0.05$), while the methane yields for R1 and R2 (ANOVA: $n = 124$; $P = 0.24$; $F = 1.40$; $\propto = 0.05$) and for R3 and R4 (ANOVA: $n = 124$; $P = 0.12$; $F = 2.46$; $\propto = 0.05$) were not statistically different between each pair of reactors. The total VFA levels, which had been ~2000 mg CH_3COOH/L for the high-ammonium digesters during the pseudo steady-state biogas production rates in period 2, decreased sharply to <1000 mg CH_3COOH/L in period 3. However, the high-ammonium reactors (R3 and R4) maintained higher VFA concentrations throughout period 3 compared to R1 and R2. Finally, during period 3, the total ammonium concentration in R3 and R4 was increased in a step-wise fashion from 4200 to 5200 mg N/L (the free ammonia increased from 200.4 to 248.2 mg N/L between days 830 and 964). We used a regression analysis and found a slightly negative slope for the methane yields when plotted over the total ammonium concentrations during the end of period 3 ($\propto = 0.05$). Thus, the methane yields were affected somewhat by increases in free ammonia concentrations at the end of the study. However, a collapse of methane generation was not observed, and therefore the threshold total ammonium concentration to inhibit methanogenesis had not been reached.

4. Discussion

4.1. *Ammonia inhibition at 25–30 °C can be alleviated by increasing the operating temperature of the digester to 35–38 °C*

We operated four digesters for almost 1000 days to investigate the interconnectivity of ammonia concentrations and operating temperature. Methane yield and VFA concentration data were used to compare high-ammonia with low-ammonia ASBRs (control reactors) treating animal waste at 25 °C and 35 °C. According to the ammonia/ammonium speciation equilibrium, increases in operating temperature will greatly raise the concentration of free ammonia, which is the relatively more important inhibiting species (El-Mashad et al., 2004; Koster and Koomen, 1988; McCarty, 1964). For example, at our constant total ammonium concentration of 4200 mg N/L and a pH of 7.65 at the end of period 2, the free ammonia concentration was 102.8 mg NH_3-N/L at 25 °C and 200.4 mg NH_3-N/L at 35 °C. Therefore, we anticipated a lower methane yield at the higher temperature due to the extensive inhibition of methanogens at this relatively high free ammonia concentration that was exceeding 200 mg N/L, which according to McCarty (1964), De Baere et al. (1984), and Hashimoto (1986) had surpassed the inhibiting ammonia threshold of 100–200 mg N/L. However, the methane yields for the high-ammonia reactors actually increased and the relative gap in yields with the low-ammonia reactors decreased from 45% to 13% when the temperature was increased. This decrease in the relative gap between the low- and high-ammonia reactors at a higher temperature can be explained by a roughly doubling of the reaction rate constant for methanogenesis for every 10 °C increase in temperature when all other environmental conditions remain constant (Banik et al., 1998; Koster and Koomen, 1988; Rittmann and McCarty, 2001). Because methanogenesis was the rate-limiting step during period 2 in R3 and R4 (elevated VFAs), a doubling of the reaction rate for methanogenesis during period 3 could alleviate the relatively higher methanogenic inhibition by free ammonia.

We found a doubling of the acetate-utilization rate constant from 0.13/day ($R^2 = 0.94$; biomass from period 2 [day 625]; free ammonia of 102.8 mg N/L) at 25 °C to 0.26/day ($R^2 = 0.99$; biomass from period 3 [day 809]; free ammonia of 200.4 mg N/L) at 35 °C of biomass from the high-ammonia reactor R4, which would have been an anticipated result if the free ammonia concentration had not risen considerably. A doubling of the kinetic constant allowed an increase in the average methane yields from 0.17 to 0.32 L CH_4/g VS fed for R3 and R4 when the temperature was increased 10 °C at these high total ammonium concentrations (Fig. 1A), but was not the only reason for this increase (see below). The total VFA concentrations for the high-ammonia reactors decreased from ~2500 to ~1000 mg CH_3COOH/L between days 719 and 760 (Fig. 1D), which consisted mainly of acetate concentrations of 2000 and 700 mg/L, respectively (Fig. 3A). Because of toxicity of acetate at concentrations of 2000 mg/L to syntrophic propionate oxidation (Fukuzaki et al., 1990; Mawson et al., 1991), we believe that the indirect benefit of lower acetate concentrations for methanogenesis is a positive feedbackward process that added to the increase in methane yields from 0.17 to 0.32 L CH_4/g VS fed even at elevated free ammonia concentrations for the 10 °C-temperature increase. Indeed, besides a decrease in acetate concentrations, we observed a relatively large decline in the propionate concentration from 550 to 140 mg/L between days 689 and 782 for R3 and R4 (Fig. 3B), which is likely due to a combination of the lower acetate concentration (and therefore lower inhibition) and a higher kinetic constant for propionate oxidation. Thus, a balance between a *decreased* toxicity to the syntrophic

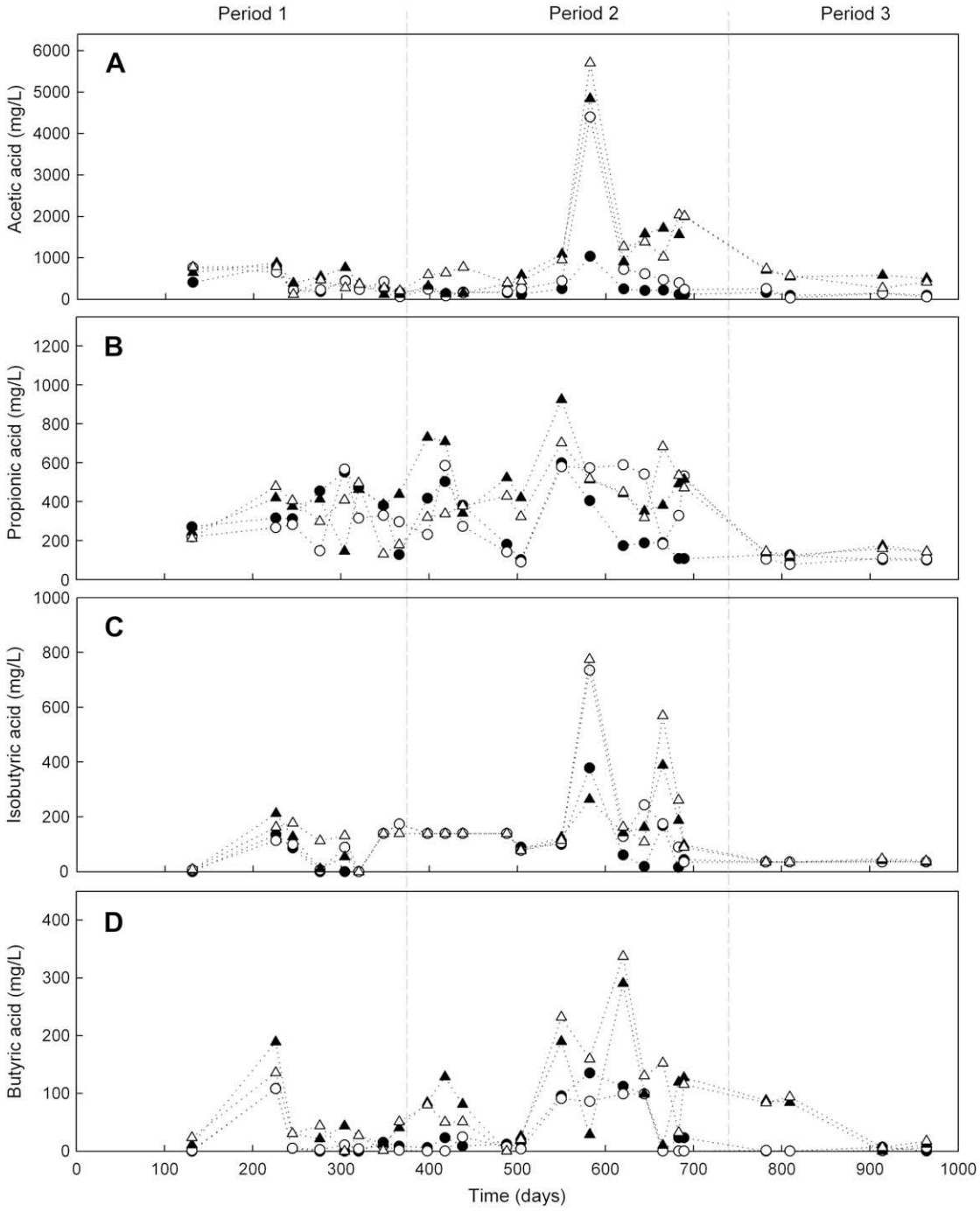

Fig. 3 – Individual volatile fatty acids data: (A) acetic acid concentration; (B) propionic acid concentration; (C) isobutyric acid concentration; and (D) *n*-butyric acid concentration. Symbols represent reactors R1 (●), R2 (○), R3 (▲), and R4 (△).

methanogenic biomass with lower acetate concentrations along with increased kinetics and an *increased* toxicity to the methanogens with higher free ammonia concentrations may partly explain the apparent lesser impact of an increased toxicity effect due to higher free ammonia concentrations.

This observation confirms the complexity of anaerobic digestion processes with several negative and positive feed-backward loops, which can make it hard to predict digester performance under changing environmental conditions. Thus far, we have discussed the following effects of the 10 °C-temperature increase on the methanogenic activity: (1) direct increase in free ammonia, resulting in a greater inhibition of methanogens; (2) direct increase in the kinetic rates for methanogenesis; (3) positive feed-backward loop of lower acetate concentrations, resulting in lower inhibition to syntrophic bacteria; and (4) direct increase in the kinetic rates for syntrophic bacteria, boosting the activity of the methanogenic consortium. In addition to these discussed effects, we observed that hydrolysis was inhibited at a constant temperature of 25 °C when the total ammonium and/or free ammonia

Table 2 – Composition of the five feed batches of swine waste used in this study.

Influent	Total ammonium-N (mg NH_4^+-N/L)	Free NH_3-N (mg NH_3-N/L)	VFA (mg CH_3COOH/L)	SCOD (mg O_2/L)	pH
Feed 1 (F1)	907	3	6500	16,610	7.5
Feed 2 (F2)	854	4	4700	12,623	7.2
Feed 3 (F3)	1261	13	3500	13,618	7.3
Feed 4 (F4)	1578	68	3650	15,100	7.6
Feed 5 (F5)	1608	74	16,000	15,983	7.9

F1–F5 were adjusted to a VS concentration of 20 g VS/L before analysis.

concentrations were increased (period 2), because the VS removal efficiencies for R3 and R4 were lower compared to R1 and R2 during period 2 (Supporting information, Fig. S2). Inhibition of hydrolysis in anaerobic digestion systems during periods of an elevated free ammonia concentration has also been observed by El-Mashad et al. (2004). During periods with high-ammonia concentrations at 25 °C, methanogenesis was the rate-limiting step with elevated acetate concentrations exceeding 2000 mg CH_3COOH/L (Fig. 3A), and the digester would have been considerably more unstable if hydrolysis had not also been inhibited. The increase in methane yields for R3 and R4 due to the 10 °C-temperature increase can, therefore, partly be explained because of a higher percentage of solids breakdown (i.e., hydrolysis), and the fact that hydrolysis became the relatively more limiting step compared to methanogenesis for these two reactors at 35 °C (i.e., methanogenesis was able to keep up with hydrolysis). This was not the only reason (as discussed earlier), since our measured doubling of the acetate-utilization rates at a high total ammonium concentration was not affected by the increase in hydrolysis (an acetate solution was supplied to biomass from R4). For the low-ammonia reactors (R1 and R2), the increase in methane yield from 0.31 to 0.36 L CH_4/g VS fed between periods 2 and 3 can be explained solely by an increase in hydrolysis (because the intermediate VFA concentrations remained similar between periods 2 and 3 in R1 and R2 [Fig. 3]). Indeed, the mean VS removal efficiencies for R1 and R2 increased from 64% to 77% between these periods with lower effluent VS concentrations at 35 °C compared to 25 °C (Supporting information: Fig. S2A).

Finally, since the kinetic constants for all microbial processes are higher, and the intermediate concentrations lower, the standard Gibbs free energy levels for all syntrophic processes are lower after the temperature increase, which will be beneficial for the anaerobic food web, and therefore methane yields. Indeed, besides the lower propionate concentrations (Fig. 3C), we observed decreasing concentrations of butyrate between days 810 and 910, indicating favorable syntrophic butyrate oxidation (Fig. 3D). Thus, an overall better functioning anaerobic food web at the higher mesophilic temperatures triggered by higher methanogenic activities explain the higher methane yields in the high-ammonia reactors even though the toxicity levels of free ammonia were higher.

Based on two sets of data points from our four reactors, we linearly extrapolated and hypothesize that at 38 °C the low- and high-ammonia reactors would generate similar methane yields (Fig. 5). This temperature coincides with the optimum temperature for mesophilic anaerobic digestion (Lettinga et al., 2001; Lindorfer et al., 2008). However, we have not verified this hypothesis and have assumed a linear behavior without experimental verification. Koster and Koomen (1988) showed that by decreasing the pH from 7.8 to 7.0 for high total ammonium concentrations at 37 °C, the ammonia inhibition was alleviated in anaerobic digesters, as anticipated by the ammonia/ammonium species equilibrium. However, this would be a costly measure on the farm due to the high alkalinity levels of digester contents. Here, we have shown that for animal manure digestion, such an effect can also be achieved when the temperature of the digester is maintained close to the optimum temperature for methanogenesis. Thus, if a farmer/operator is operating its digester in the 25–30 °C range, increasing the operating temperature to the 35–38 °C range can alleviate free ammonia inhibition. If the temperature of a heated digester is already maintained at ~35 °C, the farmer/operator should ensure not to drop the temperature during severe winter months and could alleviate ammonia toxicity problems by increasing the operating temperature to 37–38 °C.

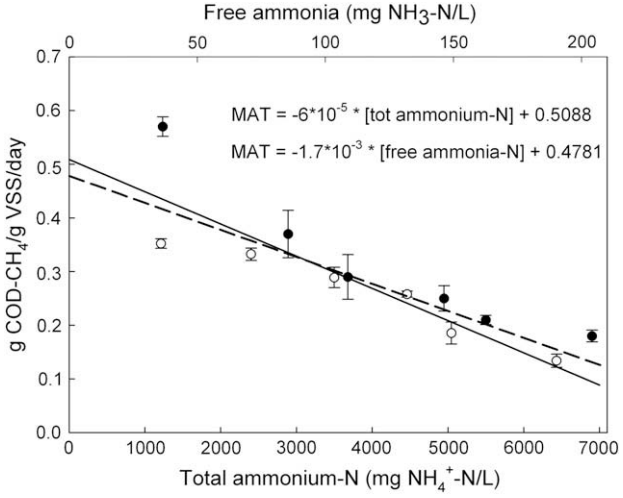

Fig. 4 – Acetoclastic methanogenic activity values in g COD–CH_4/g VSS/day at different total ammonium-N concentrations (bottom axis) and free ammonia-N concentrations (top axis; calculated values) at 25 °C at the end of period 1. Symbols represent biomass samples from R2 (●) and R4 (○). The solid line represents a linear regression of the methanogenic activity values with total ammonium-N concentrations, while the dashed line represents methanogenic activity values with free ammonia-N concentrations. The mixed liquor samples from R2 and R4 were taken on day 334 for the 1000 and 4000 mg N/L concentration; day 350 for 2000 and 5000 mg N/L; and day 370 for 3000 and 6000 mg N/L.

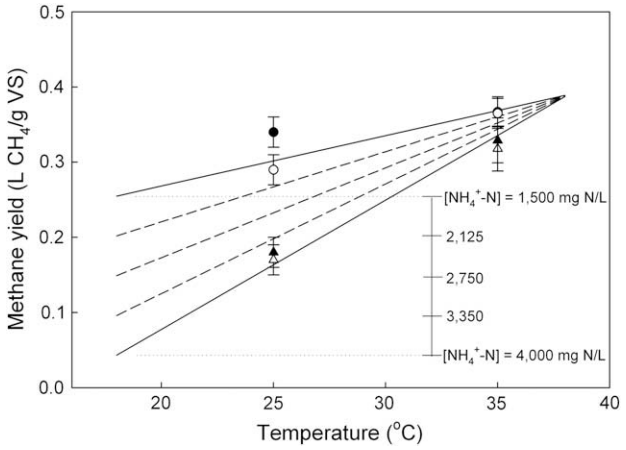

Fig. 5 – Linear extrapolation of our methane yield data from the four ASBRs during periods 2 and 3 with substrate F5 for different total ammonium-N concentrations. The top line represents linear regression of the methane yields for a total ammonium-N concentration of 1500 mg N/L; the bottom represents linear regression of the methane yields for a total ammonium-N concentration of 4000 mg N/L; while the dashed lines represent an interpolation for three intermediate total ammonium-N concentrations. Representative equations for the interaction between the total ammonium concentration and the operating temperature to calculate the methane yield (MY) for these specific conditions and total ammonium concentration ranges are: $MY = -1.2 \times 10^{-8} \times (TOT - N)^2 + 0.008 \times T + 0.12$ from 1500 to 2125 mg N/L, $MY = -4.3 \times 10^{-9} \times (TOT - N)^2 + 0.012 \times T - 0.033$ from 2125 to 2750 mg N/L, and $MY = -6 \times 10^{-9} \times (TOT - N)^2 + 0.0158 \times T - 0.134$ from 2750 to 4000 mg N/L. We assumed a linear behavior.

4.2. *The total ammonium threshold level can exceed 5200 mg N/L*

We have shown an efficient digester performance with total ammonium concentrations of ~5200 mg N/L at 35 °C. High total ammonium concentrations were also reported by Koster and Lettinga (1988) for anaerobic digesters for which total ammonium-N concentrations of 2323, 4051, 5229, and 11,831 mg N/L at 35 °C, resulting in methanogenic activities of 0.46, 0.23, 0.12, and 0.04 g COD/g VS/day, respectively. These activities were obtained from using only acetate as the substrate, and also show that the methanogenic activity is generally reduced by 50% at total ammonium-N concentration of ~4000 mg N/L (similar to our observation at 25 °C [Fig. 4]). These values show that the threshold level for successful methanogenesis can exceed 5200 mg N/L, which we confirmed here. A study on 18 full-scale anaerobic digesters revealed that ammonia was a significant factor of stability. The total ammonium-N value of 4000 mg N/L was found as the inhibitory threshold (Angelidaki et al., 2005), which is lower than for our study. However, it is difficult and probably erroneous to compare ammonium threshold levels between anaerobic digester studies given the fact that a combination of variables, such as temperature, pH levels, feed composition and variability, and bacterial and methanogenic community structure, among other operating conditions, define the inhibitory ammonia concentrations.

5. Conclusions

Throughout a period of 988 days, we operated four laboratory-scale anaerobic digesters by feeding them real swine waste at different temperatures and total ammonium concentrations while maintaining a constant pH level. We demonstrated that:

- The four bioreactors achieved a similar methane yield of 0.31 L CH_4/g VS fed under similar operating conditions at a temperature of 25 °C and a total ammonium concentration of ~1200 mg N/L.
- When the total ammonium-N levels were artificially increased to ~4000 mg N/L for two reactors (the other two reactors remained at ~1600 mg N/L), a 45% lower methane yield was observed for the high-ammonia reactors compared to the low-ammonia reactors at 25 °C.
- Increasing the operating temperature from 25 °C to 35 °C increased the methane yield in all four reactors, and resulted in a 13% lower methane yield for the high-ammonia reactors compared to the low-ammonia reactors. Direct and indirect effects of higher kinetic rates for the microbial processes at the higher temperature relieved the higher toxicity due to high free ammonia concentrations. Thus, the farmer/operator can alleviate ammonia toxicity by increasing the operating temperature when he/she is not already operating the system at the optimum temperature of 38 °C within the mesophilic range.
- At the end of the operating period, the threshold total ammonium concentration was exceeding 5200 mg N/L for the anaerobic treatment of swine waste.

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Appendix A. Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2009.02.036.

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