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ORIGINAL ARTICLE

Effect of anaerobic digestion temperature on odour, coliforms and chlortetracycline in swine manure or monensin in cattle manure*

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Abstract

Aims: This study evaluated the effect of anaerobic digestion at 22, 38 and 55°C on odour, coliforms and chlortetracycline (CTC) in swine manure or monensin (MON) in cattle manure.

Methods and Results: Swine or cattle were fed the respective growth promotant, manure was collected, and 2-l laboratory methane digesters were established at the various temperatures and sampled over 25 or 28 days. After 21 days, the concentration of CTC in the 22, 38 and 55°C swine digester slurries decreased 7, 80 and 98%, respectively. Coliforms in the 22°C digester slurries were still viable after 25 days; however, they were not detectable in the 38 and 55°C slurries after 3 and 1 days, respectively. After 28 days, the concentration of MON in the 22, 38 and 55°C cattle digester slurries decreased 3, 8 and 27%, respectively. Coliforms in the 22°C cattle digester slurries were still viable after 28 days; however, they were not detectable in the 38 and 55°C slurries after 14 and 1 days, respectively.

Conclusions: These studies indicate that anaerobic digestion at 38 or 55°C may be an effective treatment to reduce coliforms and CTC; however, it is not an effective treatment to reduce MON.

Significance and Impact of the Study: More studies are needed to determine which pharmaceuticals are susceptible to degradation by a specific manure treatment to prevent negative environmental consequences.

Introduction

The occurrence of animal pharmaceuticals in manure is considered an emerging and critical environmental issue. There is a growing concern about the impact of these pharmaceutical compounds in the manure and the effect they may have on aquatic and terrestrial organisms, and the potential development of antibiotic resistant microorganisms. Large fractions of animal pharmaceuticals are excreted into manure either as the parent compound or as a metabolite (Hamscher *et al.* 2005). In the United States, chlortetracycline (CTC) was found to be fed in approximately 55% of swine production facilities (Dewey *et al.* 1999; USDA, 2007). Dewey *et al.* (1999) found that 85% of CTC can be excreted in bioactive forms by swine. Tylosin and CTC were present in animal manure runoff when these antibiotics were included in the diets (Hoese *et al.* 2009). Greenhouse studies have indicated that corn, onions and cabbage absorb small concentrations of CTC when these plants were grown on soil where manure was applied that contained CTC (Kumar *et al.* 2005). In 1978, more than 80% of feedlot cattle were fed monensin (MON; Varel and Hashimoto 1982). This ionophore also was approved for feeding to dairy cattle in 2004 (Federal Register, 2006). With this widespread use, finding it in ground water samples on dairy farms should not be a

surprise (Watanabe *et al.* 2008, 2010). Song *et al.* (2010) found that land application of animal manures could lead to the accumulation of veterinary pharmaceuticals on crop land, and this was dependent upon timing and rate of manure application, weather conditions, soil type, landscape and crop growth. They found that MON was frequently detected in nearby surface water on a farm. Other studies have indicated between 1 and 5 mg kg^{-1} of MON in cattle faeces (Donoho 1984; Thiele-Bruhn 2003).

Thiele-Bruhn (2003) concluded that most xenobiotic compounds are degraded faster and more completely under aerobic than anaerobic conditions. Arikan *et al.* (2008) investigated the effect of composting on CTC residues in manure from medicated calves. Results from laboratory scale experiments showed that concentrations of CTC and its metabolites decreased more than 99% in 30 days in manure/straw/woodchip mixtures that reached normal composting temperatures (55°C). In comparison, concentrations of CTC and its metabolites decreased about 40% in 30 days in manure/straw/woodchip mixtures that were incubated at room temperature (25°C). In an earlier study, Arikan (2008) reported 75% degradation of CTC under 35°C anaerobic digestion conditions.

Limited studies are available on the effect of growth promotants and antibiotics in animal manure that are added to anaerobic digesters. Production of methane in anaerobic swine manure digestion was inhibited 28·4% because of the presence of CTC (Stone *et al.* 2009). When oxytetracycline and CTC were each added at 10, 50 and 100 mg l^{-1} during 35°C anaerobic digestion of swine manure, methane production was reduced 56, 60 and 62% (Álvarez *et al.* 2010). Others have found a 20% inhibition with CTC in cattle manure (35 and 55°C), and a complete inhibition of methane production when cattle manure with MON was added to digesters (Varel and Hashimoto 1981). However, the MON inhibition could be overcome after a 6-month adaptation (Varel and Hashimoto 1982). During the 1970s and 1980s, a primary objective with livestock manure was energy generation, and no efforts were made to determine the residual growth promotants and antibiotics in the manure after anaerobic digestion. Today, energy is still of interest; however, transport of pathogens and pharmaceuticals into the environment and odour reduction have overshadowed the energy issue (Miller and Berry 2005; Varel *et al.* 2008, 2010). The Food and Drug Administration (FDA) estimates that approximately 13 million kg of antibiotics were only sold for food animal production in the United States in 2009 (Food and Drug Adminstration, 2009).

The primary objective of this study was to evaluate the degradation/elimination of CTC in swine manure and MON in cattle manure after anaerobic digestion for 25–28 days. Animals were fed the respective growth promotant, manure was collected, and added to batch anaerobic digesters set at 22, 38 and 55°C. An additional objective was to compare volatile fatty acids (VFA) and aromatic compounds (collectively considered odour), coliforms and methane production from these batch digesters.

Materials and methods

Animals, diets and manure collection

Information on the 40% corn wet distillers grains (WDGS) diet that was fed to cattle in this study was provided previously (Varel *et al.* 2010). Briefly, it contained on a dry matter (DM) basis, 45% corn, 13·8% corn silage, 40% WDGS, and vitamin and mineral supplements, including MON at approximately 22 mg kg^{-1} DM. Faecal samples <24-h old were collected from cattle pens as needed to establish anaerobic digester cultures and adapt them to MON. The growing-finishing diet that was fed to swine which contained CTC at growth promoting levels (55 mg kg^{-1} DM) was previously published (Yen *et al.* 2004). It contained the following ingredients on a DM basis; 83% corn, 13% soybean meal, and vitamins and minerals as indicated in the reference. Swine manure (urine and faeces) deposited on the floor of pens prior to it passing through the slats in the floor was collected as needed to establish anaerobic digester cultures and adapt them to CTC.

Anaerobic digesters

Two-litre wide-mouth Erlenmeyer flasks with a black rubber stopper were used as the vessel for anaerobic digestion. The stopper had a 20-gauge needle inserted through it to vent the digesters. Initially to establish a methane producing culture with either swine or cattle manure, duplicate digesters at 22, 38 and 55°C were set up with a dilute slurry of the respective manure (4% DM). The manure and distilled water were blended in a Waring blender, and 600 ml of slurry was added to each digester, flushed with N_2 gas, stoppered and incubated at the respective temperature. Periodically, the digesters were sampled and analysed for VFA, pH (adjusted with NaOH) and gas composition. Once methane production was initiated and pH was stabilized in the range from 6·5 to 7 (2 and 5 months for swine and cattle manure, respectively), each digester was fed approximately 20 g of unblended manure twice per week. After 4 weeks of feeding the digesters, the contents of the duplicate digesters were mixed, and this was used as the seed culture to initiate the study, with triplicate digesters at each temperature.

The cattle manure studies were initiated by collecting enough faeces (<24-h old), blending it with distilled water

(1 : 1 ratio), and adding 300 ml of this slurry to each of 9, 2-l digesters (three replicate digesters per temperature). To each of these digesters was added 300 ml of the respective seed culture adapted to 22, 38 or 55°C. All digesters were sampled, pH was determined, and this was considered day 0. Before the digesters were gassed with N_2 and stoppered, a 50 g aliquot was removed from each digester and transferred to a 150 ml serum bottle which was gassed with N_2 and sealed. The serum bottle was used to determine methane production. Gas volume in serum bottles was determined with a glass syringe as previously described (Miller and Wolin 1974). Methane was analysed over a 25- to 28-day period using a 8610C gas chromatograph (SRI Instruments, Torrance, CA) as described by Miller and Berry (2005). A retention time up to 28 days is representative of a typical production scale anaerobic digester (Massé *et al.* 2010).

The swine studies were initiated similarly by collecting enough manure and blending it with distilled water (1 : 1 ratio), with 400 ml of this slurry added to each of 9, 2-l digesters. To each of these digesters was added 200 ml of the respective seed culture adapted to 22, 38 or 55°C. The digesters were sampled, and serum bottles were set up as indicated above for the cattle manure digesters.

All digesters were stirred before sampling at 0, 1, 3, 7, 14, 21 and 28 days. Manure slurry pH was obtained using a combination electrode and PHM 83 pH meter (Radiometer America, Cleveland, OH, USA). A 15-g sample from each digester was dried at 105°C overnight to determine DM. Another 15-g sample was acidified with 15 ml of 0·5 mol l^{-1} H_2SO_4 and stored at −20°C until analysed for fermentation products (L-lactate, acetate, propionate, isobutyrate, butyrate, isovalerate, valerate, isocaproate, caproate, phenol, *p*-cresol, indole and skatole). An autoanalyzer (Model 2700; Yellow Springs Instrument, Yellow Springs, OH, USA) was used to analyse L-lactate, and a gas chromatograph (Hewlett-Packard 6890; Agilent Technologies, Palo Alto, CA, USA) equipped with flame-ionization and mass-selective detectors was used for all other products. Conditions used for analyses of these products and ammonia have been described previously (Miller and Varel 2001; Varel *et al.* 2008). Manure slurries were separately sampled at 0, 1, 2, 4, 7, 10, 14, 21 and 25 or 28 days for the determination of coliform concentrations. A 1-g sample of manure slurry from each digester was weighed into a tube containing 9 ml of buffered peptone water (Becton Dickinson and Co., Sparks, MD, USA). Tube contents were mixed, decimally diluted in additional buffered peptone water, and plated on 3M Petrifilm *Escherichia coli* coliform count plates (3M Microbiology Products, St. Paul, MN, USA) as previously published (Varel and Wells 2007). If plates were negative for two sampling dates, no further samples were analysed. A 10-g sample was obtained from the flasks and immediately frozen for determination of CTC or MON.

Determination of chlortetracycline

To 1 g (approximately 1 ml) of swine manure, 9 ml of phosphate buffered saline (PBS, pH 7·2) was added, and the sample was vortexed and centrifuged at 1300 ***g*** for 5 min. The supernatant was transferred to a 50 ml tube, and the original tube was rinsed twice with 5 ml of PBS which was combined with the supernatant. After centrifuging at 1300 ***g*** for 5 min, the supernatant was removed and centrifuged at 18 000 ***g*** for 10 min. The supernatant was further diluted 1 : 10 with PBS for use in the ELISA analysis.

A Ridascreen tetracycline ELISA kit (R-Biopharm AG, Darmstadt, Germany) was adapted for swine manure ELISA analysis similar to a previously described approach (Shelver *et al.* 2008). This kit was selected because of its previous demonstration for the analysis of CTC in swine manure (Kumar *et al.* 2004) and the low cross-reactivity for normal degradation (metabolism) products of CTC [4-epi-chlortetracycline (4-epi-CTC) 1·5%, 4-epi-anhydrochlortetracycline (4-epi-anhydro-CTC) 0·1%, iso-chlortetracycline (iso-CTC) 0·2% relative to tetracycline (data provided by R-Biopharm AG) making this assay very specific for CTC. A composite control swine manure (CTC-free) at 1 : 200 dilution was spiked with CTC at 0, 0·15, 0·45, 1·35, 4·05 and 12·15 ng ml^{-1} to generate the standard curve. The procedure provided by the manufacturer was followed for the remainder of the assay. Briefly, 50 μl of standard or diluted swine manure and 50 μl of anti-tetracycline antibody were added to the tetracycline-protein-conjugate wells, and the mixture was incubated at room temperature for 1 h. The plate was washed three times with PBS amended with 0·05% Tween 20 (PBST). One hundred microliters of peroxidase conjugated secondary antibody solution was added and incubated at room temperature for 15 min. The plate was washed three times with PBST, and 100 μl of chromogen was added and incubated at room temperature for 15 min after which 100 μl of stop solution was added and the absorbance read at 450 nm using a Bio-Rad microplate reader (model 550; Bio-Rad Laboratories, Inc. Hercules, CA, USA). The calibration curve was fitted with a logistic curve fit function using Microplate Manager™ (ver. 4.0; Bio-Rad Laboratories, Inc.), and unknown concentrations were computed from the calibration curve as CTC equivalent. If the CTC concentration of the treatment sample exceeded the working range, the samples were further diluted and re-assayed. All samples were analysed in triplicate wells per assay, with the assay being repeated on three separate days. The characteristics of the assay were

obtained by fitting the standard curve with the four parameter logistic equation and using the fitted parameters to compute the 10% inhibition of absorbance at 0 ng ml^{-1} (the limit of detection) and the 20 and 80% inhibition concentrations (the working range). The average limit of detection was 0·40 ng ml^{-1} (n = 12), and the average working range was 0·8–9·0 ng ml^{-1} (n = 12). An independent estimate of the detection limit based on the mean ±3 SD from control manures (n = 21) gave a value of 0·27 ng ml^{-1} in good agreement with the 0·40 ng ml^{-1} from the standard curve data. The mean recovery of CTC spiked into manure at 0·5, 1, 2 and 4 ng ml^{-1} ranged from 78 to 88% with inter-day % CV ranged from 5 to 20% (n = 3). The intra-day variation for levels of 1 and 4 ng ml^{-1} was 18 and 15%, respectively, with recoveries of 90 and 75%, respectively (n = 8).

Determination of monensin

Sample preparation for LC/MS-MS analysis.
Monensin was extracted from the cattle manure using a variation of the method described previously (Sassman and Lee 2007). Each cattle manure sample (10 ml of slurry) was spiked with 100 μl of a 10 mg l^{-1} salinomycin solution in acetonitrile as a surrogate standard to monitor recovery. Efficiency of recovery for MON was determined using 10 ml samples of control slurry manure prepared from animals fed diets without MON that were spiked with 100 μl solutions of 10 mg l^{-1} MON and salinomycin each. The manure samples were transferred to individual 50 ml centrifuge tubes for solvent extractions (2 : 1 ratio of solvent to extract) using a two-step extraction with 20 ml of acetonitrile followed by 20 ml of 50 : 50 acetonitrile:ethyl acetate solution. After each solvent addition, the sample was mixed for 1 min using a vortex mixer, centrifuged (5 min at 1200 ***g***), and the upper extraction layers were decanted into glass beakers which were allowed to evaporate off the solvent in a hood (approximately 2 days). Following evaporation, the samples were dissolved in 40 ml of an acetonitrile : water mixture (50 : 50 v/v). Each sample (1·5 ml) was transferred to amber autosampler vials and analysed for MON and salinomycin by liquid chromatography (LC) mass spectrometry.

LC/MS-MS analysis for monensin.
Samples were analysed using a Waters 2695 LC and Micromass Quattro Ultima MS with an electrospray source using an XBridge C18, 5 μm 2·1 × 150 mm column from Waters in conjunction with an XBridge C18 guard column. Positive ionization modes were used for detection. A gradient separation was utilized involving a mixture of solvent A (70 : 30 1% formic acid:methanol) mixed with solvent B (100% methanol). LC conditions were set to 0·250 ml min^{-1} flow rate, column temperature of 45°C and injection volume was either 2 or 10 μl. The column gradient profile started with 50% solvent A and 50% solvent D at 0 time and programmed linearly in 8 min to 7% solvent A and 93% solvent D, where this mixture was maintained for 15 min then returned quickly (1 min) to initial conditions and allowed to equilibrate here for 9 min. The analytes were detected using multiple reaction monitoring methods available on the triple quadrupole mass spectrometer, with transition mass selections as follows: MON, 693·8 > 675·9 m/z and salinomycin at 773 > 430·9 m/z (Sekar and Wu 2006; Sassman and Lee 2007). Recoveries of ionophores in spiked blank manure samples were as follows: 72% MON and 76% salinomycin. There was an insignificant level (< 0·1% of treatment levels) of MON present in the blanks that appeared to be possible background levels. A surrogate standard (salinomycin) was used to monitor recovery of each sample, which ranged from 31 to 130% in all the samples with an average of 79% overall. Recoveries of the surrogate standard were lowest for the 22°C group (58% *vs* 80–100% for the other two groups) suggesting that matrix interference may have been a factor with these because organic matter was highest in this group of samples. Quantification was performed using the external standard method using four point standard calibration curves (fits with >0·90 r^2 values). Results were not corrected for recovery or blank levels.

Statistical analyses

Data for incubations were analysed as a split-plot in time with incubation flask as the experimental unit. An initial model was used with all interactions, and after step-down analyses, the final model included the effects of incubation flask, treatment, time and incubation flask nested within dietary treatment. Treatment was tested with incubation flask nested within treatment as the error term. Bacterial numbers were transformed to $\log_{10}$ colony-forming unit (CFU) per gram of wet weight before statistical analysis. When dietary treatment × time interaction was observed, differences among means were tested with a protected t-test. For all statistical analyses, differences were considered significant when the probabilities were <0·05. Data are presented as means of three or four replicate digesters with standard errors. Statistical analyses were conducted using the GLM procedure (SAS Inst. Inc., Cary, NC, USA).

Results

In an initial study, when manure slurries (from swine fed CTC or no CTC) were incubated at 22°C, there were no

differences ($P > 0{\cdot}05$) in the production of total VFA (odor), methane or pH over 25 days (Fig. 1). Also, little CTC was degraded/eliminated; 6·5, 5·8 and 6·0 mg l^{-1} CTC was present at 0, 7 and 21 days, respectively, as indicated below. Therefore, it was decided that a mesophilic and thermophilic temperature, 38 and 55°C, respectively, should be evaluated. Different species of micro-organisms are present in digesters run at these higher temperatures.

A comparison of the straight-chained VFA, branched-chained VFA and aromatic compounds from 22, 38 and 55°C digesters that contained swine manure slurries (DM 12·5–13·5%) with CTC is shown in Fig. 2. In general, straight- and branched-chain VFA increased rapidly between 1 and 7 days for all three temperatures (Fig. 2a–f). Slurries in the 22°C digesters had the lowest VFA concentrations, with 38°C digester slurries having the highest concentrations with the exception of butyrate (Fig. 1c). The 55°C digester slurry concentrations were somewhere in between the 22 and 38°C. The aromatic compound, *p*-cresol, was also greatest in the 38°C digester slurries, with the lowest concentration found in the 55°C digester slurries (Fig. 1g). The 55°C digester slurries contained the greatest phenol concentrations (Fig. 2i).

Data in Fig. 3 indicate pH, methane production, ammonia, lactate, coliform concentrations and degradation/elimination of CTC in these digesters. With the exception of the 55°C digesters, pH initially decreased from days 0 to 1, then continuously increased over 25 or 28 days, especially with the 22°C digesters, whereas the 38 and 55°C digester slurries levelled off after approximately 7 and 14 days, respectively (Fig. 3a). Methane production was highest from the slurries in the 38°C digesters, lowest in the 55°C digesters, with an intermediate level from the 22°C slurries (Fig. 3b). Ammonia and L-lactate concentrations were highest with the 38°C digester slurries, and few differences were observed between the 22 and 55°C slurries (Fig. 3c,d). As expected, no coliforms were detected in the 55°C manure slurries after day 0, none were detected in the 38°C slurries at day 3; however, coliforms were still present in the 22°C slurries at day 25 (Fig. 3e). Minimal concentrations of CTC disappeared in the 22°C slurries between day 0 (6·5 mg l^{-1}) and day 21 (6·0 mg l^{-1}; Fig. 3f). Contrary to this, in the 38 and 55°C slurries most of the CTC disappeared (day 0, 8·3 mg l^{-1} compared to day 28, 1·7 mg l^{-1}; and day 0, 5·9 mg l^{-1} compared to 1·0 mg l^{-1}, respectively, for 38 and 55°C).

A comparison of the straight-chained VFA, branched-chained VFA and aromatic compounds from 22, 38 and 55°C digesters which contained cattle manure slurries (6·0–6·5% DM for 38 and 55°C, and 10% DM for 22°C digesters) with MON is shown in Fig. 4. All of these compounds, with the exception of butyrate (Fig. 4c) and isovalerate (Fig. 4f), were greatest in the 22°C digester slurries, presumably because of the higher initial DM in these digesters. If we had mixed all three temperature seed cultures, we may have obtained more similarities in these parameters. The 55°C digester slurries had the greatest concentration of butyrate throughout, and isovalerate after day 7. However, the aromatic compounds, *p*-cresol, indole and phenol were generally the lowest in the 55°C slurries (Fig. 4g,h,i).

Data in Fig. 5 indicate pH, methane production, ammonia, lactate, coliform concentrations and degradation/elimination of MON in these digesters. The pH of the slurries in all digesters initially dropped between days 0 and 1, with the 38°C slurries declining the most, down to 6·43. However, after day 1 pH continued to increase in the 38°C slurries until the study was terminated at

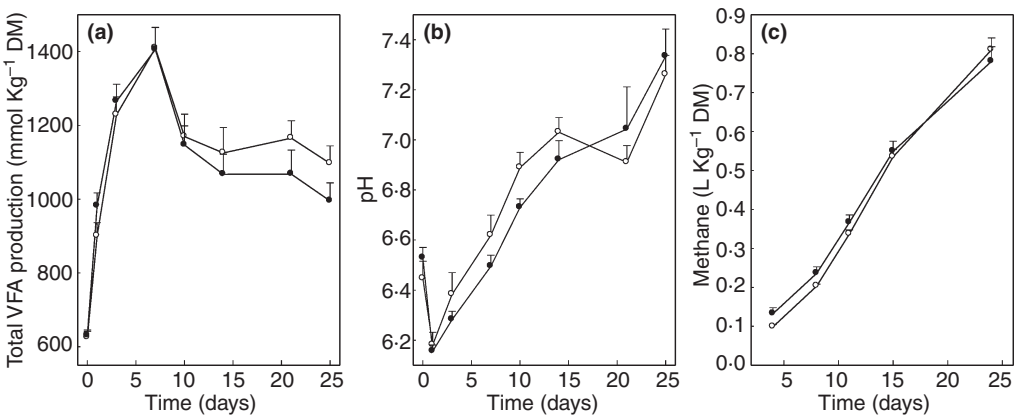

Figure 1 Production of total volatile fatty acids, methane and pH values from 22°C batch anaerobic digesters that were fermenting swine manure with or without chlortetracycline in the diet. Means represent four replicate digesters with standard error bars. Symbols: ○ no chlortetracycline; ● chlortetracycline.

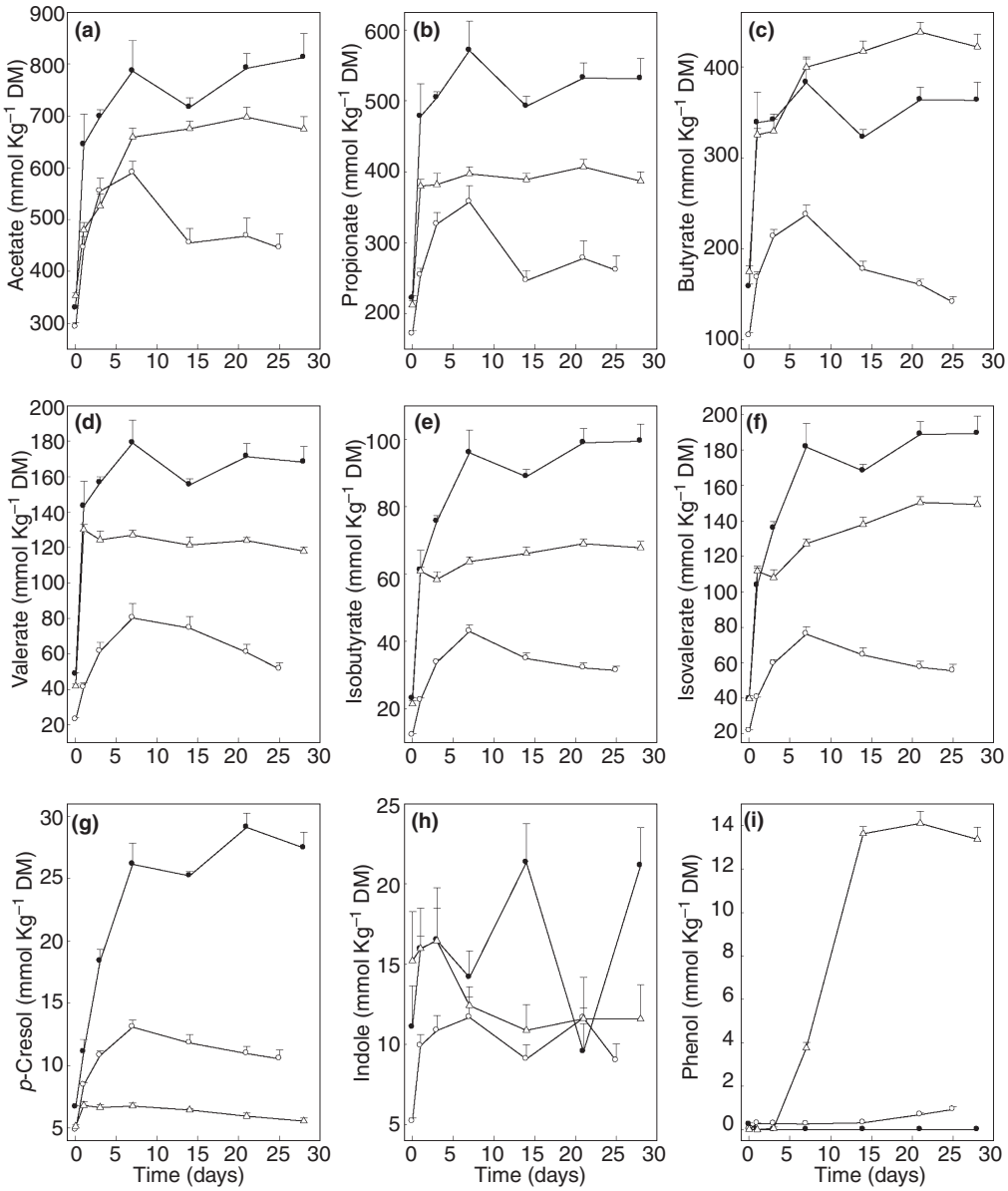

Figure 2 Accumulation and degradation of individual and branched-chained volatile fatty acids, and aromatic fermentations products from 22, 38 and 55°C batch anaerobic digesters that were fermenting swine manure with chlortetracycline in the diet. Means represent three replicate digesters with standard error bars. Symbols: ○ chlortetracycline (22°C); ● chlortetracycline (38°C); △ chlortetracycline (55°C).

28 days (Fig. 5a). The pH of the 55°C slurries rose between days 7 and 14; however, by day 14 again dropped to 6·3 at which time they were adjusted to pH 7·0 with NaOH. A proportional amount of NaOH was injected into the respective serum bottles. The 55°C slurries then remained above 6·5 for days 21 and 28. The 22°C slurries fell to pH 6·75 at day 7, remained there until day 14 and rose slightly above 6·8 by days 21 and 28. Methane production was greatest from the slurries in the 38°C digesters, lowest in the 22°C digesters, with an intermediate level from the 55°C slurries (Fig. 5b). Ammonia and L-lactate concentrations were initially and throughout the 28 days, greatest in the 22°C slurries (Fig. 5c,d). Ammonia concentrations in the 38 and 55°C slurries were roughly similar to day 7, with the 38°C slurries increasing more rapidly than the 55°C slurries. L-lactate concentrations increased rapidly in the 38°C slurries to day 14; however, none surpassed those of the 22°C slurries, while those in the 55°C slurries were lowest amongst the three slurries (Fig. 5d). Again as expected,

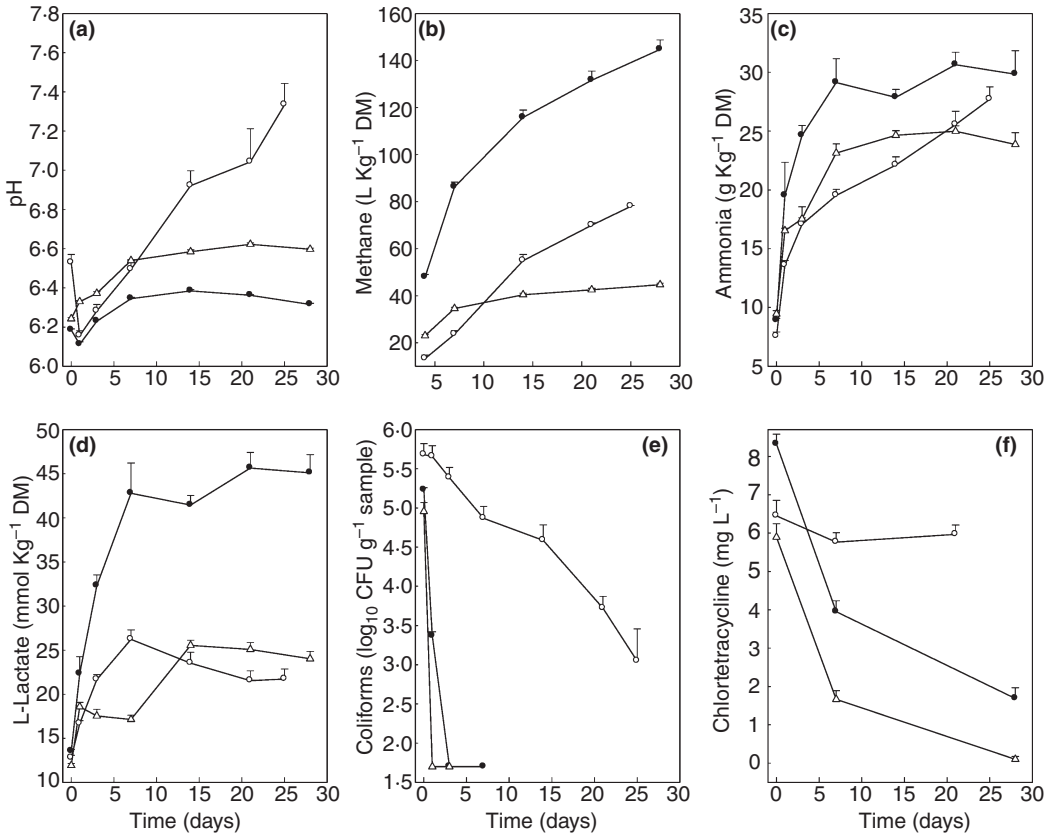

Figure 3 pH, methane, ammonia, L-lactate, degradation/elimination of chlortetracycline and survival of coliforms from 22, 38 and 55°C batch anaerobic digesters that were fermenting swine manure with chlortetracycline in the diet. Means represent three replicate digesters with standard error bars. Symbols: ○ chlortetracycline (22°C); ● chlortetracycline (38°C); △ chlortetracycline (55°C).

no coliforms were detected in the 55°C manure slurries after day 0 (Fig. 5e), and none were detected in the 38°C slurries at day 14. However, similar to the swine manure digesters, coliforms were still present in the 22°C slurries at day 25. Concentrations of MON were unchanged in the 22°C slurries between day 0 (0·74 mg l^{-1}) and day 28 (0·72 mg l^{-1}; Fig. 5f). In the 38 and 55°C slurries, respectively, slightly more of the MON disappeared (day 0, 0·36 mg l^{-1} compared to day 28, 0·33 mg l^{-1}; and day 0, 0·30 mg l^{-1} compared to day 28, 0·22 mg l^{-1}; Fig. 5f).

Discussion

This study indicates that CTC in swine manure did not affect ($P > 0·05$) total VFA (potential odor) or methane production from 25 days batch anaerobic digesters incubated at 22°C (Fig. 1a,c). If the concentrations of VFA and aromatic compounds (Fig. 2a–i) were added together and considered potential odour (Varel *et al.* 2008, 2010), the least odour would come from a 22°C digestion of the swine manure, followed by a 55 and 38°C digestion.

This study also indicates that little CTC (7%) is degraded/eliminated at 22°C under anaerobic conditions during a 21-days incubation period (Fig. 3f). Kumar *et al.* (2004) demonstrated that the ELISA kit used for the determination of CTC was very sensitive, specific and could be applied to manure samples for the measurement of CTC. Furthermore, the manufacturer demonstrated low cross-reactivity to the normal degradation products of CTC, epi-CTC, epi-anhydro-CTC and iso-CTC. Kumar *et al.* (2004) noted ELISA and LC-MS gave comparable results although ELISA determination gave slightly higher (approximately 30%) than those obtained from LC-MS. The specificity of the ELISA was demonstrated by Kumar *et al.* (2004) showing both ELISA and LC-MS giving no CTC levels on both control samples and one environmental sample. In our study, the four 22°C control digester samples showed no detectible change in CTC concentration. Therefore, we conclude that a higher temperature or longer incubation period will be required to metabolize CTC. When the incubation temperature of the methane digesters with CTC in swine manure was increased to 38 and 55°C, the CTC disappeared by 80 and 98%,

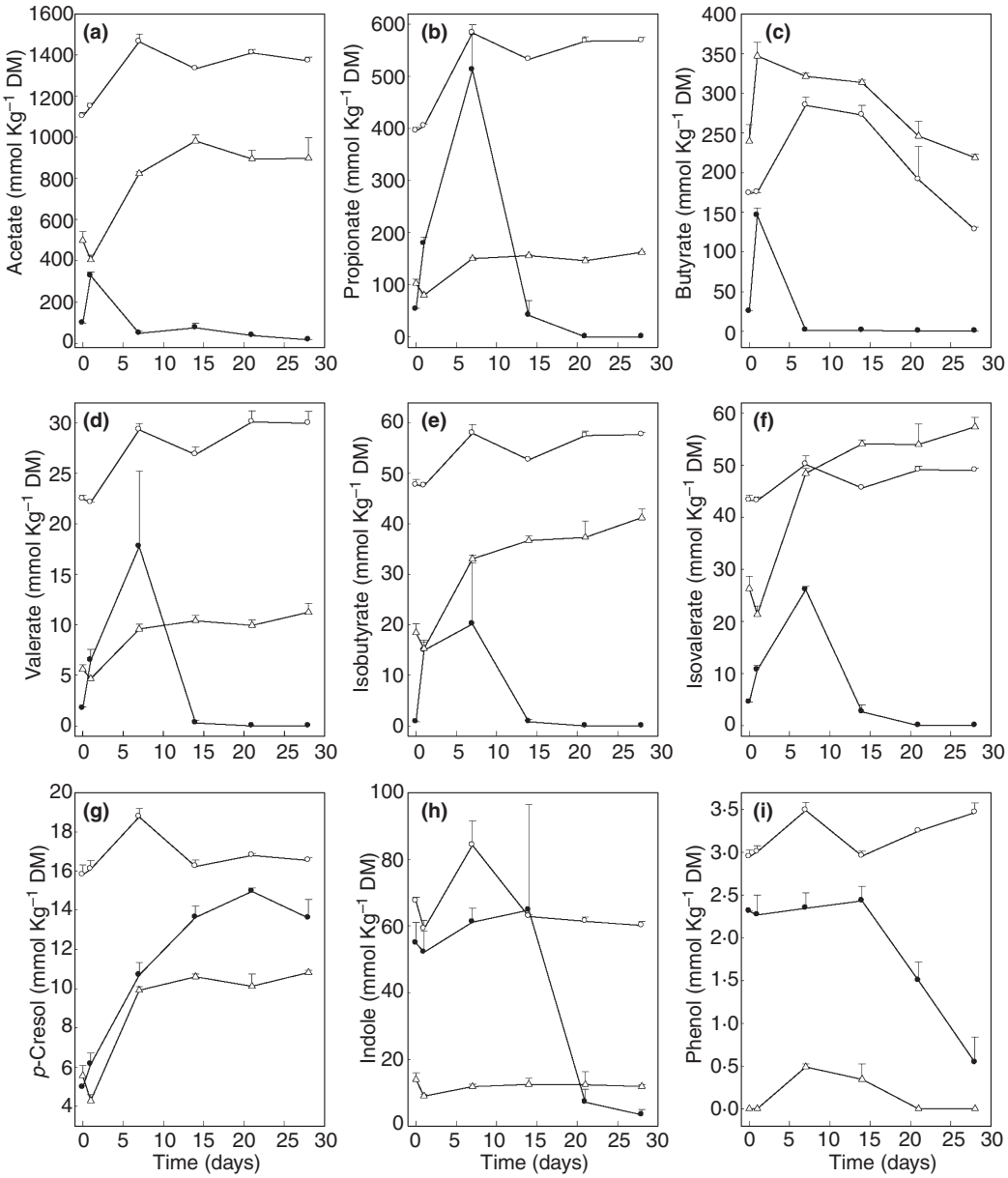

Figure 4 Accumulation and degradation of individual and branched-chain volatile fatty acids, and aromatic fermentations products from 22, 38 and 55°C batch anaerobic digesters that were fermenting cattle manure with monensin in the diet. Means represent three replicate digesters with standard error bars. Symbols: ○ chlortetracycline (22°C); ● chlortetracycline (38°C); △ chlortetracycline (55°C).

respectively, after 28 days (Fig. 3f). Besides the disappearance of CTC at temperatures above 22°C, the coliforms were eliminated from the digester slurries by days 1 and 3, respectively, at 38 and 55°C. Elimination of the coliforms and the majority of CTC in the 38 and 55°C digesters are two advantages that should be considered, in addition to the energy generated, when contemplating a treatment for swine manure. Other additional benefits from the use of anaerobic digesters for manure treatment are the conversion of VFA to methane (reduction of odour), and the fertilizer value or plant essential nutrients (N, P and K) are retained in the digester effluent (Massé *et al.* 2010). Unfortunately, with an aerobic treatment of the manure, much of the N can be lost to emission.

Álvarez *et al.* (2010) found a 56% reduction of methane production in 35°C batch culture assays when 10 mg l^{-1} each of oxytetracycline and CTC were added to swine manure. They also reported that 90% of the CTC concentration was degraded after 21 days. Arikan (2008) indicated 75% of CTC was degraded in manure from

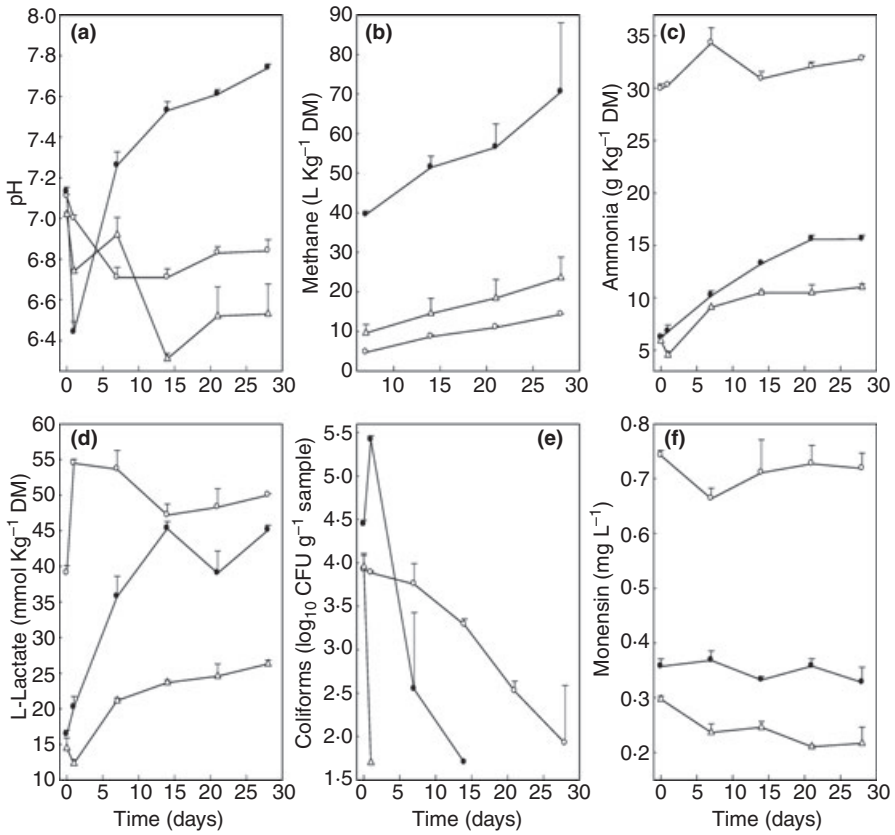

Figure 5 pH, methane, ammonia, L-lactate, degradation/elimination of monensin and survival of coliforms from 22, 38 and 55°C batch anaerobic digesters that were fermenting cattle manure with monensin in the diet. Means represent three replicate digesters with standard error bars. Symbols: ○ chlortetracycline (22°C); ● chlortetracycline (38°C); △ chlortetracycline (55°C).

manure of medicated calves which had been subjected to 33 days of anaerobic digestion at 35°C. Massé *et al.* (2010) studied the potential of low-temperature (15–25°C) anaerobic digestion on swine manure containing six different antibiotics: tylosin, lincomycin, tetracycline, sulfamethazine, penicillin and carbadox. They indicated that process stability was not affected by the presence of antibiotics in the manure, although penicillin and tetracycline reduced methane production by 35 and 25%, respectively. They also concluded that low-temperature bioreactors may be more tolerant to high ammonia concentrations than mesophilic (38°C) or thermophilic (55°C) bioreactors, because the ratio of free (NH_3) to total ($NH_3 + NH_4$) ammonia decreases with temperature. Unfortunately, they did not evaluate the fate of the antibiotics in their bioreactors. The initial concentration of CTC in our 38°C slurries was greater ($P < 0{\cdot}05$), 8·3 mg l^{-1} compared to 6·5 and 5·9 mg l^{-1}, respectively, for the 22 and 55°C slurries. This may explain why the majority of the concentrations of VFA and branched-chain VFA in these slurries are greater ($P < 0{\cdot}05$; Fig. 2a,b,d–f). Normally, as the temperature increases, microbial metabolism increases; thus, one would expect a more rapid production of methane from the higher temperature. This was true when the 38°C slurries are compared to the 22°C slurries. However, the 55°C digester slurries produced the lowest methane volume of the three temperatures. This is contrary to what might be expected, unless CTC is somehow inhibiting the fermentation.

Unlike the results from CTC in swine manure, if the concentrations of VFA and aromatic compounds (Fig. 4a–i) were added together and considered potential odour, the most odour would come from a 22°C digestion of the cattle manure and the least odour would come from a 38°C digestion. Also MON was more resistant to degradation than CTC under the conditions used in this study. After 28 days, only 27% of MON disappeared at 55°C (Fig. 5f). Possibly a longer retention time than 28 days would affect this outcome; however, this may preclude using anaerobic digestion as a practical solution for reducing antibiotics from release into the environment. Storteboom *et al.* (2007) determined the half-life of MON to be 14·7–30·1 days, and 5·1–8·4 days for CTC, depending upon the management of the manure. Carlson

and Mabury (2006) indicated that MON dissipation half-life was found to be much shorter in the field (3–4 days) than in a controlled laboratory study, perhaps, because of differences in microbial communities. Aerobic conditions in the field may explain the short half-life. The half-life of MON under anaerobic manure conditions were reported to be >70 days (Thiele-Bruhn 2003). In general, these studies have found that antibiotic dissipation/degradation kinetics depend on characteristics of the particular antibiotic such as water solubility and soil sorption capacity and on environmental conditions such as temperature, light, pH and oxygen levels (Thiele-Bruhn 2003; Storteboom *et al.* 2007). Our results of limited MON degradation/elimination at 55°C suggests anaerobic digestion is not an efficient method to degrade MON. Similarly, this may explain the difficulty in establishing steady-state anaerobic digesters with manure that contains MON. Our earlier studies (Varel and Hashimoto 1981, 1982), and the current one, indicated that a 5- to 6-month adaptation period was necessary to acclimatize the micro-organisms to MON, whereby steady-state conditions could be established to produce methane from manure containing MON. Two other ionophores, lasalocid and salinomycin, had minimal effects on the rate of methane production (Varel and Hashimoto 1982); therefore, in future studies, the degradation of these two ionophores should be evaluated in anaerobic digesters.

In summary, this study indicates that CTC in swine manure can be reduced by 80 and 98%, respectively, through the use of 38 and 55°C anaerobic digesters. However, 22°C anaerobic digesters only removed 7%. A more recalcitrant antibiotic was MON, with only 27% breakdown in a 55°C digester, and even less (8%) in a 38°C digester. Thus, as a previous study has suggested (Mohring *et al.* 2009), prevention of antibiotics in the environment may involve selecting an antibiotic that is susceptible to degradation by anaerobic digestion or another manure treatment. Anaerobic digesters offer several advantages as a manure treatment, including elimination of pathogens, reduction of odour, generation of energy and retention of all essential fertilizer nutrients in the digester effluent.

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