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ScienceDirect
Journal of Hazardous Materials 158 (2008) 485–490
Journal of Hazardous Materials
www.elsevier.com/locate/jhazmat

Degradation and metabolization of chlortetracycline during the anaerobic digestion of manure from medicated calves

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Received 7 October 2007; received in revised form 28 January 2008; accepted 29 January 2008
Available online 7 February 2008

Abstract

The fate of antibiotic residues in the manure of treated animals is of considerable concern because of the potential development of antibiotic-resistant bacteria in the environment. The objective of this study was to determine the fate of chlortetracycline (CTC) during the anaerobic digestion of manure from medicated calves. Five beef calves were medicated for 5 days with 22 mg/kg/day of CTC. Manure samples collected from calves after medication were diluted 5-fold with water, loaded into triplicate 1 L anaerobic digesters and incubated at 35 °C. The CTC concentration decreased approximately 75% (from 5.9 to 1.4 ppm) during the 33 days digestion period, yielding a half-life of about 18 days. The concentration of the CTC epimer, 4-epi-chlortetracycline (ECTC), declined roughly 33% (from 4.1 to 2.5 mg/L) during anaerobic digestion. However, the concentration of the CTC metabolite, iso-chlortetracycline (ICTC), increased 2-fold (from 2.3 to 4.6 mg/L) during the digestion period. Although the water-soluble concentration of CTC decreased 84% (from 0.3 to 0.04 mg/L), the water-soluble concentrations of ECTC and ICTC increased roughly 2-fold during digestion (from 0.5 to 0.93, and 1.0 to 2.7 mg/L, respectively). Since ECTC and ICTC are more water-soluble than the parent tetracycline CTC, it is more likely that these compounds present in digested manure slurry will be detected in water monitoring samples.

Keywords: Chlortetracycline; Anaerobic digestion; Manure; Antibiotic; Fate; Calf

1. Introduction

Chlortetracycline (CTC) is a broad-spectrum antibiotic used for prophylactic and therapeutic use in poultry, pigs and calves. Although the use of antibiotics as growth promoters has been banned since January 2006 in the European Union, CTC is one of only ten antibiotics licensed in the U.S.A. for use as growth promoters for livestock [1]. 4-Epi-chlortetracycline (ECTC) is an epimer of CTC and iso-chlortetracycline (ICTC) is an isomer of CTC (Fig. 1). The fate of these compounds in treated swine has been reported by Grote et al. [2]. Elmund et al. showed that approximately 75% of ingested CTC was recovered in manure [3].

The release of antibiotics into the environment is of considerable concern because persistent antibiotic residues may lead to the development of antibiotic-resistant bacteria [4]. The widespread use and relative persistence of CTC have lead to its detection in soil [5] and surface waters [6]. Hamscher et al. detected average concentrations of 9.5 μg/kg CTC in the upper 10 cm of the soil from eight fields that had been manured with animal slurry 2 days before sampling [5]. Concentrations decreased with depth to about 0.7 μg/kg below 80 cm. In a subsequent study, CTC levels between 0.17 and 0.22 μg/L were found in water samples collected at 80 and 120 cm depth by the same researchers. CTC was found in 2.4% of the 84 surface water samples with the maximum concentration of 0.69 μg/L by Kolpin et al. [6]. They speculated that the low frequency of CTC detections in water samples was likely due to the hydrophobic nature of tetracyclines and that such compounds would be more likely to be present in stream sediments than in stream water.

Anaerobic digestion is an established technology for the treatment of animal manure. Although a number of investigators have studied the fate of antibiotics in soil interstitial water [7], and in anaerobic lagoons [8,9] there is very limited information on the fate of CTC during anaerobic digestion of manure. The fate of oxytetracycline in manure from medicated calves during manure digestion was recently studied [10]. The objective of this study

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doi:10.1016/j.jhazmat.2008.01.096

was to determine the fate of CTC during the anaerobic digestion of manure from medicated calves.

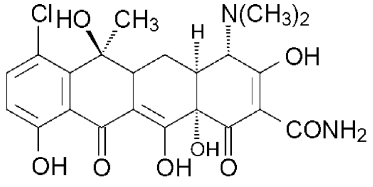
2. Materials and methods

2.1. Chemicals

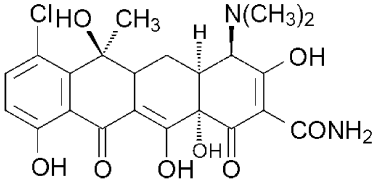
Chlortetracycline hydrochloride (M_w = 515, CAS no. 64-72-2), 4-epi-chlortetracycline hydrochloride (M_w = 515, CAS no. 101342-45-4), iso-chlortetracycline hydrochloride (M_w = 515, CAS no. 89835-80-3), and demeclocycline hydrochloride (M_w = 502, CAS no. 64-73-3) were purchased from Acros Organics N.V. (Fair Lawn, NJ). Stock solutions of these compounds (100 mg/L) were prepared in methanol and stored in the dark at −20 °C. They were diluted with methanol to obtain standard solutions in the range of 0.01–10 mg/L. HPLC-grade methanol was obtained from Fisher Scientific (Fair Lawn, NJ). All others reagents used in this study were of analytical grade. The water used in the experiments was purified by using reverse osmosis and activated carbon. McIlvaine buffer (pH 4) was prepared by mixing aqueous solutions of 0.1 M citric acid and 0.2 M disodium hydrogen phosphate (62:38, v/v). Methanolic oxalic acid (0.01 M) was prepared by dissolving oxalic acid in methanol.

2.2. Animal medication and sample collection

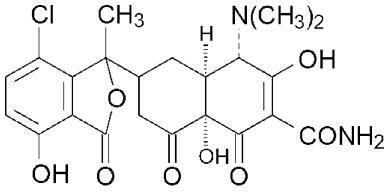
Five male and female beef calves, 5–7 months old and ranging from 190 to 350 kg in body mass, were kept in individual pens in a beef barn. Pens were scraped clean daily, after which approximately 2 kg of sawdust was scattered on the floor of each pen as bedding material. After a 2-week acclimatization period for the animals, the manure–sawdust mixture from each pen was collected, pooled, mixed, and the mixture was stored at 4 °C until later use as the "unmedicated manure". The calves were then medicated for 5 days at 22 mg/kg body mass per day of CTC (a standard dosage in agricultural practice; [11]) by ingestion of the daily ration containing CTC as a feed additive. Feed consisted of a mixture of beef creep pellet (31%), corn silage (43%), and grass silage (26%). Medicated grain was given to the animals prior to other constituents in order to insure complete consumption of the CTC dose. Medicated manure–bedding mixtures collected on the fifth day of medication were combined and used in laboratory experiments as the "medicated manure".



Chlortetracycline (CTC)



4-epi-chlortetracycline (ECTC)



Iso-chlortetracycline (ICTC)

Fig. 1. Chemical structures of CTC, its epimer (ECTC) and isomer (ICTC).

Table 1
Characteristics of diluted medicated-high and medicated-low manure slurries used in anaerobic digesters (mean ± S.E.)

Constituent	Medicated-high manure slurry	Medicated-low manure slurry
pH	7.6 ± 0.1	7.6 ± 0.1
Total alkalinity, mg $CaCO_3$/L	2,950 ± 29	3,137 ± 96
Total solids, mg/L	47,000 ± 2000	40,000 ± 3000
Volatile solids, mg/L	42,150 ± 1600	35,500 ± 2700
Ammonium-N, mg/L	640 ± 20	570 ± 2
Chemical Oxygen Demand, mg/L	52,240 ± 3900	49100 ± 4560
Buffer extractable CTC, mg/L	5.86 ± 0.34	0.98 ± 0.08
Water-soluble CTC, mg/L	0.32 ± 0.03	0.02 ± 0.01
Buffer extractable ECTC, mg/L	4.11 ± 0.41	0.56 ± 0.09
Water-soluble ECTC, mg/L	0.49 ± 0.03	<MDL
Buffer extractable ICTC, mg/L	2.36 ± 0.23	0.28 ± 0.02
Water-soluble ICTC, mg/L	1.03 ± 0.08	0.05 ± 0.02

MDL: Method detection limit.

2.3. Anaerobic digestion

Anaerobic digestion experiments were carried out batch-wise in six laboratory digesters (Bellco Biotechnology, NJ, US) each with a working volume of 1 L. Medicated manure from the fifth day of antibiotic treatment was diluted 5-fold with tap water to approximately 5% total solids (a level that is representative of digester influent in commercial farm operations; [12]), and 800 mL of manure slurry was loaded into each three digesters (referred to in this study as medicated-high digesters). The pH and water hardness values of the tap water were 7.5 and 65 mg/L, respectively. In order to determine the fate of lower CTC concentrations, the manure from medicated calves was first diluted 5-fold with unmedicated manure, then the mixture was diluted 5-fold with tap water to approximately 5% total solids, and 800 mL of manure slurry was loaded into the remaining three digesters (referred to in this study as medicated-low digesters). 200 mL of effluent from a dairy manure digester was added to each digester as inoculum. Table 1 shows characteristics of the diluted medicated-high and medicated-low manure slurries that were loaded into the digesters. After the digesters were filled, the headspaces were flushed with nitrogen gas to remove traces of oxygen. The digesters were stirred continuously to avoid compaction and incubated at a mesophilic

temperature (35 ± 1 °C). Manure slurry samples (30 mL) were collected from each digester on days 0, 12, 23 and 33 and analysed for CTC, ECTC and ICTC. Manure slurry samples were collected using 50 mL syringe under nitrogen gas. Digesters were stirred continuously in order to collect homogeneous samples during the sample collection. Levels of pH, total solids (TS), volatile solids (VS), total alkalinity, ammonium-N and chemical oxygen demand (COD) were determined at the beginning of the study.

TS, VS and total alkalinity were determined according to APHA [13]. Ammonium-N was determined colorimetrically by flow injection analysis (Lachat Instruments, Milwaukee, WI). Hach COD Reactor (digestion at 150 °C for 2 h) and a Hach spectrophotometer were used for chemical oxygen demand (COD) analyses.

Removal of compounds was assumed to follow first-order kinetics. A rate constant, k, was determined as the slope of the curve calculated by linear regression. The half-life, $t_{1/2}$, was then calculated as $t_{1/2} = \ln(2)/k$.

2.4. Extraction of CTC, ECTC and ICTC

Concentrations of water-soluble and buffer extractable CTC, ECTC and ICTC in manure slurry samples were determined in duplicate. To determine water-soluble concentrations of CTC, ECTC and ICTC, 5 mL manure slurry samples were subjected to centrifugation (7000 × g, 20 min, 5 °C), after which 0.5 mL supernatant and 0.5 mL water were vortexed for 30 s in a separate tube. The mixture was transferred to a 2 mL amber autosampler vial, and 20 μL of demeclocycline was added as an internal standard. For buffer extractable concentrations of CTC, ECTC and ICTC, the method described by Capone et al. was used [14]. Briefly, 2 mL samples were extracted three times with 3 mL of 0.1 M Na_2EDTA-McIlvaine buffer (pH 4) by vortexing for 30 s followed by sonication for 5 min in a 100 W sonication bath (Bronson Ultrasonics, Danbury, CT). After each extraction, the extracts were subjected to centrifugation (500 × g, 5 min, 5 °C), the supernatants were pooled, again subjected to centrifugation (7000 × g, 20 min, 5 °C), filtered through Whatman glass microfiber (grade GFB) filter paper, and passed through prewashed Waters 60-mg HLB (hydrophilic–lipophilic balance) Oasis® cartridges (Waters Corp., Milford, MA). The cartridges were prewashed with 5 mL of methanol followed by 10 mL of 0.1 M Na_2EDTA-McIlvaine buffer. After the extracts were loaded, the cartridges were flushed with 20 mL distilled water, followed by sample elution using 8 mL of 0.01 M methanolic oxalic acid. The eluents were concentrated under a flow of N_2 to a volume of 0.5 mL by evaporation. Then, 0.5 mL water was added to the tube, and the tube was vortexed for 30 s. The resulting mixture was transferred to 2 mL amber autosampler vials, and 20 μL of demeclocycline was added as an internal standard prior to analysis by LC–MS/MS.

2.5. LC–MS/MS analysis

The analyses of CTC, ECTC and ICTC were performed using LC–MS/MS. The LC instrument was a Waters 2690 XE (Waters Corp.) separations module with an Xterra MS C_{18} column (150 mm × 2.1 mm i.d., 5 μm) (Waters Corp.) at 45 °C; the injection volume was 10 μL. A mobile-phase gradient was necessary to separate the compounds. The respective compositions of solvents A, B and C were as follows: (A) 1% formic acid–methanol (70:30, v/v); (B) water, and (C) methanol. The solvents were mixed as follows: 0–1 min, 50% (A) 50% (B) 0% (C); 1–12 min, a linear gradient from the previous settings to 70% (A) 0% (B) 30% (C); 12–20 mi,n 42% (A) 0% (B) 58% (C); 20–22 min, 0% (A) 0% (B) 100% (C); 22–25 min, 0% (A) 0% (B) 100% (C), and finally the instrument was returned to starting conditions from 25 to 27 min and then allowed to stabilize for 10 min with 50% (A) 50% (B). The total run time was 37 min. The flow rate was set at 0.25 mL/min. Six-point calibration curves were generated using results from 10 μL injections of standards ranging from 0.01 to 10 mg/L. The method detection limits (MDL) were estimated based on the injections of the lowest concentrations of the standard solutions and the signal-to-noise (S/N) = 3/1 criterion was applied. The minimum level of quantitation (ML) was calculated as 3.18 times the MDL [15]. The ML for CTC, ECTC and ICTC were 0.04 mg/L. Atmospheric pressure ionization-tandem mass spectrometry was performed on a benchtop triple quadrupole mass spectrometer (Quattro LC from Micromass Ltd., Manchester, U.K.) operated in electrospray ionization mode. The source parameters were as follows: capillary voltage was set at 3.5 kV and extractor voltage was set at 3 V, respectively; rf lens at 0.1 V; source and desolvation temperatures were 150 and 450 °C. Liquid nitrogen was used to supply the nebulizer and desolvations gas (flow rates were approximately 80 and 600 L/h, respectively). Argon was used as collision-induced decomposition gas to fragment the parent ions; the typical pressure was 2.6×10^{-3} mbar. Both high and low mass resolutions were set at 12.0 for both quadrupoles. Acquisition was done in the multiple-reaction monitoring mode (MRM) in electrospray negative (ES−). The parent and daughter ions used for compound identification and quantitation are listed in Table 2 along with the optimum cone voltages and collision energies used. Optimization was performed by infusion of the standards from a syringe pump (10 μL/min) mixed with the LC effluent (100% A; 200 μL/min), with high and low-mass resolution set at 15.0. Detector was a photomultiplier set at 650 V. Analyte concentrations were calculated by the internal standard method using demeclocycline as an internal standard [16]. To determine demethylation of CTC to demeclocycline, control samples were spiked CTC, ECTC and ICTC without demeclocycline. Demeclocycline appearance was not observed

Table 2
Parent and daughter ions used for quantitation of CTC, ECTC and ICTC and MS parameters used to produce them

Compound	Parent ion (Da)	Daughter ion (Da)	Retention time (min)	Cone (V)	Collision (eV)
CTC	477	392	10.2	32	23
ECTC	477	392	8.1	32	23
ICTC	477	197	8.5	35	19
Demeclocycline	463	378	8.5	27	18

suggesting demethylation of CTC to demeclocycline did not occur during the analyses. Peak integration and quantitation were performed automatically using the MassLynx 3.5 software (Waters Corp.).

2.6. Determination of extraction efficiencies for CTC, ECTC and ICTC

To determine extraction efficiencies, duplicate samples of unmedicated manure slurry were spiked at 0.5 and 5 mg/L for CTC, ECTC and ICTC, incubated 30 min, and extracted as described above. Recovery results shown in Table 3 were calculated as a means of duplicate samples at each concentration. Recoveries of 0.5 mg/L spikes for CTC, ECTC and ICTC were higher than recoveries of 5 mg/L spikes. Average recoveries of CTC, ECTC and ICTC were about 90, 85 and 78%, respectively.

Table 3
Recovery of CTC, ECTC and ICTC in manure slurry[a]

Compound	Recovery (% mean ± S.D.) Spike level (mg/L)	
	0.5	5.0
CTC	92 ± 4	88 ± 8
ECTC	89 ± 6	81 ± 6
ICTC	79 ± 4	77 ± 8

[a] Recovery values are the means from duplicate samples.

3. Results and discussion

Anaerobic digesters were incubated for 33 days at 35 °C using diluted manure collected from calves after CTC medication. The levels of buffer extractable and water-soluble CTC, ECTC and ICTC during anaerobic digestion are shown in Fig. 2. The buffer extractable CTC levels decreased from initial values

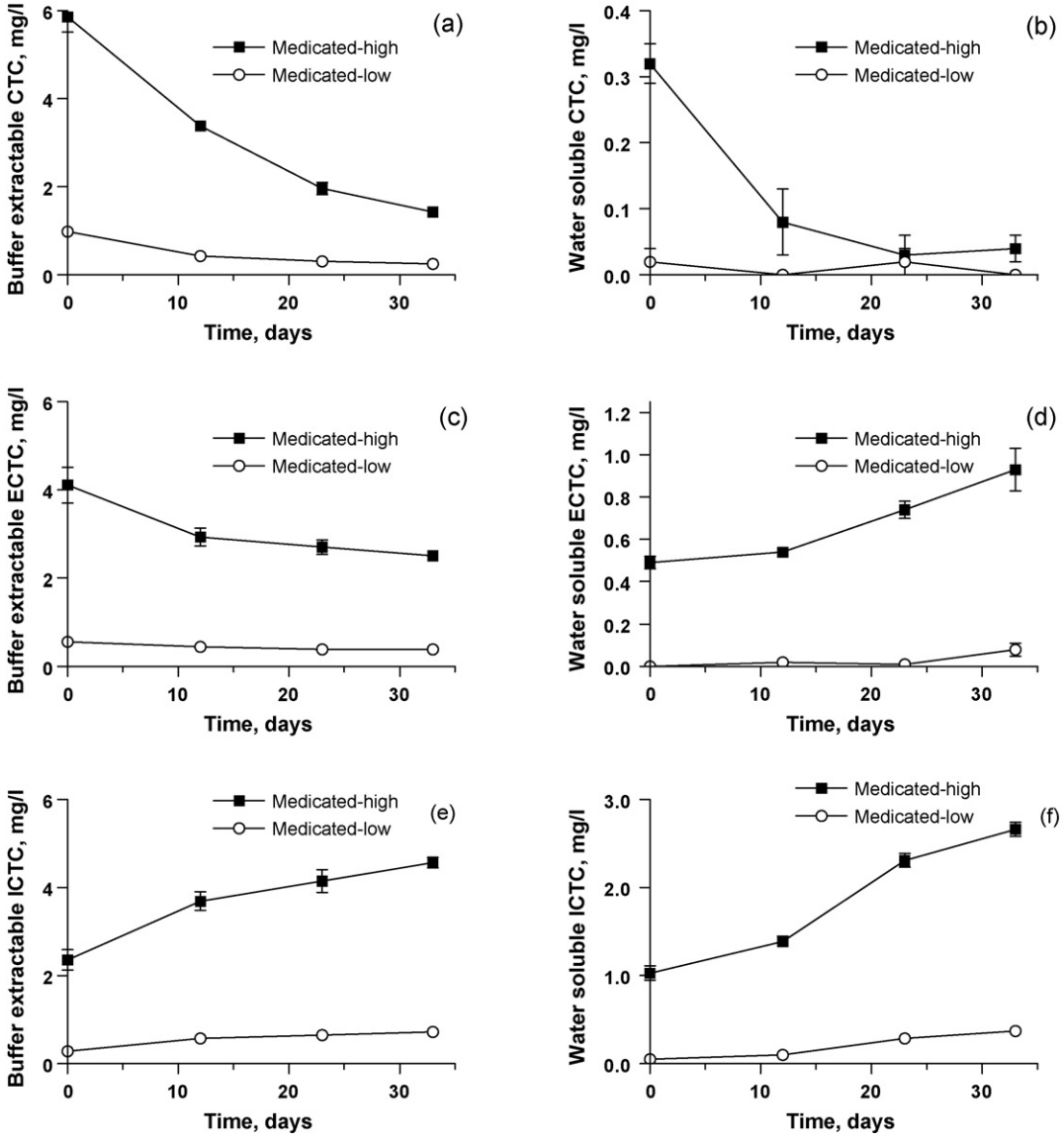


Fig. 2. Buffer extractable and water-soluble concentrations of CTC, ECTC and ICTC during anaerobic digestion of medicated calf manure slurry. Values are the means from triplicate digesters. Standard errors are shown as error bars. Method detection limits for CTC, ECTC and ICTC were 0.01 mg/L.

of 5.9 ± 0.3 and 1.0 ± 0.1 to 1.4 ± 0.1 and 0.3 ± 0.1 mg/L at the end of the study, for medicated-high and low digesters, respectively (Fig. 2a). Overall, 74 and 75% removal of buffer extractable CTC were achieved for medicated-high and low digesters, respectively, during 33 days of anaerobic digestion, yielding an average calculated CTC half-life value of 18 days. The water-soluble CTC levels decreased from 0.32 ± 0.03 to 0.04 ± 0.02 mg/L (a 84% reduction, half-life value of 12 days) within 33 days for medicated-high digesters (Fig. 2b). The initial water-soluble CTC concentrations were 0.02 ± 0.01 mg/L for medicated-low digesters and decreased to under the method detection limit (0.01 mg/L) by day 33. When water-soluble and buffer extractable CTC levels were compared, it was found that only about 5% of CTC was water-soluble. Winckler and Grafe reported a 55–57 day tetracycline half-life value using 20 and 100 μg/mL spiked concentrations in pig slurry incubated at 8 °C. They also reported a 105-day half-life value using 20 μg/mL spiked concentration in outdoor experiments [17]. In contrast, Kühne et al. reported half-life values of only 4.5 and 9 days at ambient temperature for tetracycline in aerated and non-aerated pig manure (containing 200 μg/mL tetracycline), respectively [18]. In our recent study, we determined oxytetracycline half-life value of 56 days for 10 mg/L initial concentration using calf manure at 35 °C [10].

For medicated-high digesters, buffer extractable ECTC levels decreased gradually from 4.1 ± 0.4 mg/L at the start of experiment to 2.5 ± 0.1 mg/L on day 33, yielding a calculated half-life value of 39 days, while water-soluble ECTC concentrations increased from 0.5 ± 0.03 mg/L on day 0 to 0.93 ± 0.1 mg/L on day 33 (Fig. 2c and d). For medicated-low digesters, buffer extractable ECTC levels decreased from 0.56 ± 0.09 mg/L at the start of experiment to 0.39 ± 0.04 mg/L on day 33, yielding a calculated half-life value of 50 days, while water-soluble ECTC concentrations increased from under the detection limit (0.01 mg/L) on day 0 to 0.08 ± 0.03 mg/L on day 33.

The buffer extractable ICTC levels increased gradually from initial 2.4 ± 0.2 and 0.28 ± 0.02 to 4.6 ± 0.1 and 0.72 ± 0.03 mg/L at the end of the study, for medicated-high and low digesters, respectively (Fig. 2e). Similarly, water-soluble ICTC concentrations also increased from initial 1.03 ± 0.1 and 0.05 ± 0.02 to 2.66 ± 0.2 and 0.37 ± 0.03 mg/L at the end of the study, for medicated-high and low digesters, respectively (Fig. 2f).

ECTC and ICTC were present in the manure from medicated calves. Therefore, both medicated-high and low digesters had some initial concentrations of these compounds. Although buffer extractable ECTC levels declined during anaerobic digestion, buffer extractable and water-soluble ICTC concentrations increased for both medicated-high and low digesters. In addition, water-soluble ECTC levels increased during anaerobic digestion for both medicated-high and low digesters. These results agree with a previous study in which decreases in CTC and ECTC levels and an increase in the level of ICTC were observed over time in outdoor anaerobic pig lagoons [19].

A study regarding the sorption of CTC showed that ICTC and ECTC distributed more towards the water phase ($K_{d,\,ICTC} < K_{d,\,ECTC} < K_{d,\,CTC}$) compared with the parent CTC, suggesting that the mobility of these compounds was higher than of the parent compound [20]. This suggests that the ECTC and ICTC present in digested manure slurry, although expected to be less potent than the parent compound [21] will more likely to be detected in water rather than the parent compound.

4. Conclusions

Approximately 75% removal of buffer extractable CTC was achieved in 33 days by anaerobic digestion at 35 °C yielding a calculated value half-life of about 18 days. Although levels of buffer extractable ECTC declined during anaerobic digestion, concentrations of buffer extractable and water-soluble ICTC increased for both medicated-high and low digesters. In addition, levels of water-soluble ECTC increased during anaerobic digestion for both medicated-high and low digesters. Since ECTC and ICTC are more water-soluble than the parent tetracycline CTC, it is more likely that these compounds present in digested manure slurry will be detected in environmental water monitoring samples.

Acknowledgement

The author is grateful to veterinarian Dr. William Hare for conducting animal feeding study.

References

[1] R.A. Swick, Role of growth promotants in poultry and swine feed, Am. Soybean Assoc. Tech. Bull. (1996), AN04:10.
[2] M. Grote, A. Vockel, D. Schwarze, A. Mehlich, M. Freitag, Fate of antibiotics in food chain and environment originating from pigfattening (Part 1), Fresenius Environ. Bull. 13 (11b) (2004) 1216–1224.
[3] G.K. Elmund, S.M. Morrison, D.W. Grant, M.P. Nevins, Role of excreted chlortetracycline in modifying the decomposition process in feedlot waste, Bull. Environ. Contam. Toxicol. 6 (2) (1971) 129–132.
[4] J.C. Chee-Sanford, R.I. Aminov, I.J. Krapac, N.G. Garrigues-Jeanjean, R.I. Mackie, Occurence and diversity of tetracycline resistance genes in lagoons and groundwater underlying two swine production facilities, Appl. Environ. Microbiol. 67 (2001) 1494–1502.
[5] G. Hamscher, A. Abu-Quare, S. Sczesny, H. Höper, H. Nau, Determination of tetracyclines and tylosin in soil and water samples from agricultural areas in Lower Saxony, in: L.A. van Ginkel, A. Ruiter (Eds.), Proceedings of the Euroside IV Conference, National Institute of Public Health and the Environment (RIVM), Bilthoven, Netherlands, 2000, pp. 522–526.
[6] D.W. Kolpin, E.T. Furlong, M.T. Meyer, E.M. Thurman, S.D. Zaugg, L.B. Barber, H.T. Buxton, Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: a national reconnaissance, Environ. Sci. Technol. 36 (2002) 1202–1211.
[7] T. Søeberg, F. Ingerslev, B. Halling-Sørensen, Chemical stability of chlortetracycline and chlortetracycline degradation products and epimers in soil interstitial water, Chemosphere 57 (2004) 1515–1524.
[8] K.A. Loftin, C. Henny, C.D. Adams, R. Surampali, M.R. Mormile, Inhibition of microbial metabolism in anaerobic lagoons by selected sulfonamides, tetracyclines, lincomycin, and tylosin tartrate, Environ. Toxicol. Chem. 24 (2005) 782–788.
[9] A.C. Kolz, T.B. Moorman, S.K. Ong, K.D. Scoggin, E.A. Douglass, Degradation and metabolite production of Tylosin in anaerobic and aerobic swine-manure lagoons, Water Environ. Res. 77 (2005) 49–56.
[10] O. Arikan, L.J. Sikora, W. Mulbry, S.U. Khan, C. Rice, G.D. Foster, The fate and effect of oxytetracycline during the anaerobic digestion of manure from medicated calves, Process Biochem. 41 (2006) 1637–1643.

[11] K. Bennett, Compendium of Veterinary Products, second ed., North American Compendiums Inc., 1993.
[12] A. Wilkie, Anaerobic digestion of dairy manure: design and process considerations, in: Proceedings of the Dairy Manure Management Conference, Natural Resource, Agriculture and Engineering Service, Cornell University, Ithaca, NY, 2005, pp. 301–312.
[13] American Public Health Association (APHA), Standard Methods for the Examination of Water and Wastewater, 18th ed., Washington, DC, 1992.
[14] D.G. Capone, D.P. Weston, V. Miller, C. Shoemaker, Antibacterial residues in marine sediments and invertebrates following chemotherapy in aquaculture, Aquaculture 145 (1996) 55–75.
[15] U.S. Environmental Protection Agency. Guidelines establishing test procedures for the analysis of pollutants (App. B to Part 136, Definition and procedure for the determination of the method detection limit) U.S. Code of Federal Regulations, Title 40, revised as of July 1, 1992.
[16] J. Zhu, D.D. Snow, D.A. Cassada, S.J. Monson, R.F. Spalding, Analysis of oxytetracycline, tetracycline, and chlortetracycline in water using solid-phase extraction and liquid chromatography tandem mass spectrometry, J. Chromatogr. A 928 (2001) 177–186.
[17] C. Winckler, A. Grafe, Use of veterinary drugs in intensive animal production: evidence for persistence of tetracycline in pig slurry, J. Soil Sed. 1 (2001) 66–70.
[18] M. Kühne, D. Ihnen, G. Möllller, O. Agthe, Stability of tetracycline in water and liquid manure, J. Vet. Med. A 47 (2000) 379–384.
[19] M. Meyer, Pharmaceuticals and other emerging contaminants in US water resources: methods, results, and a developing national perspective, in: Workshop to identify emerging contaminants of concern and their implications for the estuarine and human health, Washington DC, USA, May 11–12, 2005.
[20] J. Tjørnelund, R.B. Jensen, H.P. Ma, B. Halling-Sørensen, Sorption of chlortetracycline and its decomposition products in soil, Dans Kemi 81 (2000) 12–14.
[21] B. Halling-Sørensen, G. Sengeløv, J. Tjørnelund, Toxicity of tetracyclines and tetracycline degradation products to environmentally relevant bacteria including selected tetracycline resistant bacteria, Arch. Environ. Contam. Toxicol. 42 (2002) 263–271.