



Original article

Impact on degradation of antibiotics from poultry litter using Autothermal Thermophilic Aerobic Digestion (ATAD)



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ABSTRACT

Tetracycline (TC) is one of the common antibiotics which is widely used in livestock growth promotion. The prevalent application TC may pave way to progression of antibiotic resistant bacteria. The main objective of this study is to determine the effect of Autothermal Thermophilic Aerobic Digestion (ATAD) on the fate of TC residues found in digested poultry litter. For the determination of TC in poultry litter, thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) were done. TLC result revealed that the R_f value of standard TC on TLC plate was 0.97 which correlates with the R_f value of TC at 0, 12, 24 and 36 h of digested poultry litter sample and not at 48, 60 and 72 h. HPLC chromatogram revealed that the limits of detection and the recovery were 5 µg/kg and 96% for standard TC. Linear correlation curves were obtained over the series of 100–500 µg/mL with correlation coefficient of 0.996 and the calibration curve was $Y = 0.001X + 0.066$. These results confirmed the degradation of TC in ATAD digestion of poultry litter by abiotic processes.

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1. Introduction

In global scenario, the emergence and dissemination of antibiotic resistant human pathogens is of foremost concern (Herath et al., 2016) which is directly connected to the usage of antibiotics in veterinary (Ferber, 2000; Fey et al., 2000; Mund et al., 2017). About 17–76% of the administered antibiotics are expelled in unaltered forms as metabolites through urine and feces (Jjemba, 2002; Suresh et al., 2018). In food products, the amount of antibiotics and antibiotic resistance genes (ARG) is more/ less whereas in animal manure the density of antibiotic resistant bacteria is high (van

den Bogaard and Stobberingh, 2000). Animal waste, namely poultry litter contains high level of protein, nitrogen and other minerals including phosphorus, potassium and calcium. Hence it can be used in farming as an organic fertilizer (Kelleher et al., 2002; Sridevi Dhanarani et al., 2016). In addition, it also contains heavy metals, hormones and antimicrobial compounds. From animal husbandry, the antibiotic residues enter the environment directly by spreading of poultry litter or storage in the form of sludge (Martínez-Carballo et al., 2007). Excessive land application of poultry litter leads to accumulation of antibiotic residues which cause surface and ground water pollution (Cao et al., 2020; Chee-Sanford et al., 2001). The development of antibiotic resistant bacteria is through run off and leaching where they find impact on both human health and environment (Boxall et al., 2003; Khojasteh et al., 2018).

Antibiotics in poultry litter have to be treated in order to prevent its adverse effect on human and the environment (Søberg et al., 2004). Antibiotic resistant microorganisms in poultry litter have been reported in our previous studies (Dhanarani et al., 2009). During anaerobic digestion of manure, antibiotics such as

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chlorotetracycline (CTC) in anaerobic lagoons (Kolz et al., 2005; Lofin et al., 2005) are degraded into iso-chlorotetracycline (ICTC) which is less potent than the parent compound (CTC). The oxytetracycline (OTC) from the excreta of medicated calves was highly reduced in its digestion (Arikan, 2008; Arikan et al., 2006). Hence, ATAD is an applicable method for the degradation of tetracycline in poultry litter.

A sensitive method has to be developed to determine the concentration of antibiotics in ecological samples such as solid waste, wastewater, etc (Cheng et al., 2018; Liu et al., 2018). Numerous chromatographic assays have been recognized for the determination of tetracycline in environmental samples (Arikan et al., 2007; D'Angelo and Martin, 2018; Jacobsen et al., 2004). LC-MS are preferred due to their high sensitivity and information about the compound confirmation, but are deliberated to be costly. GC-MS is not appropriate for most of the antibiotics due to their volatility (Malintan and Mohd, 2006). HPLC has been successfully used to determine tetracycline in animal product (Arikan et al., 2006; Wen et al., 2006) and food samples (Oka et al., 2000). The main goal of this study is to determine the fate of tetracycline using TLC and HPLC during Autothermal Thermophilic Aerobic Digestion (ATAD) of poultry litter.

2. Materials and methods

2.1. Detection of tetracycline using TLC

Approximately 10 g of poultry litter was ground in 10 mL of ethanol. The suspension was transferred to falcon tube and centrifuged (7000 rpm for 10 min). The clear supernatant was taken in a separate tube and evaporated using N₂ stream. It was then dissolved in 0.2 mL methanol (Tajick et al., 2002). For the preparation of silica plates, about 2 g of silica powder was mixed thoroughly to produce fine paste with 5 mL of distilled water. Clean glass plates were coated with silica paste by TLC gel spreader system to 0.25 mm depth and plates were stimulated at 120 °C for 2 h (Boyer, 1993). About 5–10 µL of methanol extracts were spotted on silica plates by fine glass capillaries. Treated plates were placed in tank having acetone-methanol (1:1) as the mobile phase. When the solvent front has reached to the bottom of the plate, chromatograms were detected by placing the plate on iodine vapor. Standard was done by dissolving 0.1 g of tetracycline in 4 mL of methanol (Thangadurai et al., 2002).

2.2. Detection of tetracycline using HPLC

Approximately 0.1 g of poultry litter sample was added to 5 mL of ethyl acetate, sonicated (20 min) and kept for 30 min. The ethyl acetate (3 mL) supernatant was removed to a centrifuge tube and evaporated using nitrogen. The deposit was then thawed in 3 mL of 20% (v/v) methanol comprising 4% of sodium chloride solution and filtered (0.45 µm Millipore membrane) prior injection to the HPLC system (Shimadzu UFLC 12AS HPLC). Standard was made by dissolving 500 µg of tetracycline in 1 mL of methanol (Wang et al., 2008). Samples (50 µL) were injected onto a C-18, 4.6 mm × 250 mm (i.d. 5 µm) column. The mobile phase A contained of 25% (v/v) acetonitrile and mobile phase B consisted of 0.01 mol/L NaH₂PO₄ and 0.001 mol/L of Na₂EDTA adjusted to pH 2.5 using nitric acid at room temperature. The flow rate of mobile phase was 1.0 mL/min. The detection wavelength was optimized at 270 nm. Calibration graphs were run using standard tetracycline solution. The quantity of standard was plotted within the linear portion of the calibration graph and it was from 100 to 500 µg/mL. The linearity was assessed using linear regression analysis, which was calculated by the least square regression method and

it was found to be 0.998 for standard. The reproducibility of the HPLC finding, expressed as relative standard deviation (RSD) was 9.3% and their detection limits was 0.035 mg/L.

3. Results and discussion

3.1. Thin layer chromatography

Chromatography on thin layer of silica gel resulted in migration of tetracycline. The R_f value obtained for standard tetracycline was 0.97. The digested poultry litter at 0, 12, 24 and 36 h showed similar migration when chromatographed with acetone-methanol (1:1) of relatively high polarity. The R_f value of samples at 0, 12, 24 and 36 h were found to be 0.99, 0.99, 0.97 and 0.98, respectively (Fig. 1). The R_f value of the above samples had clear correlation with standard when compared at 48, 60 and 72 h. The digested poultry litter at 48, 60 and 72 h showed that the sample remained almost at the point of application and there was no migration of sample when chromatographed with solvents of relatively high polarity. This is due to low concentration of tetracycline at 48, 60 and 72 h of digested poultry litter than at 0, 12, 24 and 36 h of digested poultry litter. This method is easy to perform, precise and cost effective so that it can be implemented certainly in laboratories, but minus is its accuracy when compared to HPLC and it has reported noticeable antibiotics in 50% of chicken meat samples (Abdel-Mohsein et al., 2015; Tajick and Shohreh, 2006).

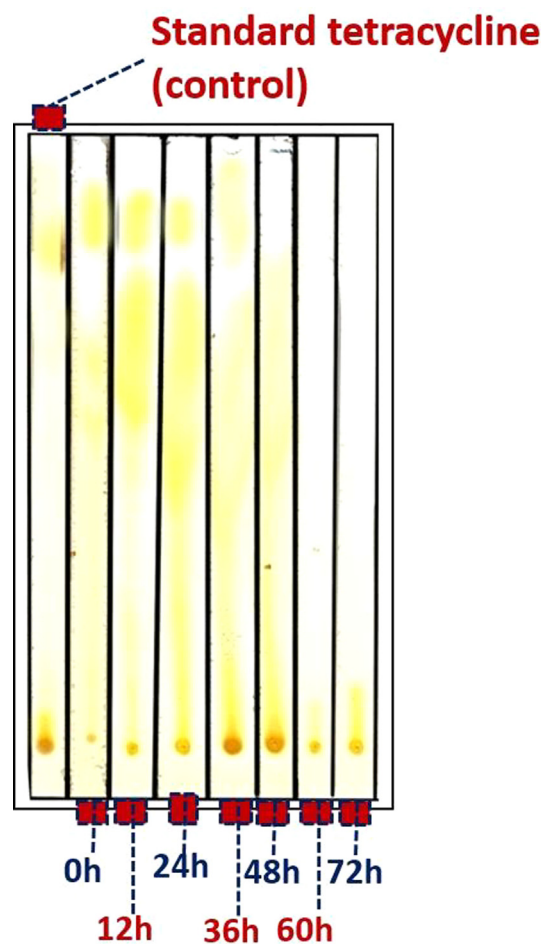


Fig. 1. Thin layer chromatogram of tetracycline. S- Standard tetracycline, Lanes 1–7 – ATAD digestion of poultry litter at different digestion time (0, 12, 24, 36, 48, 60 and 72 h).

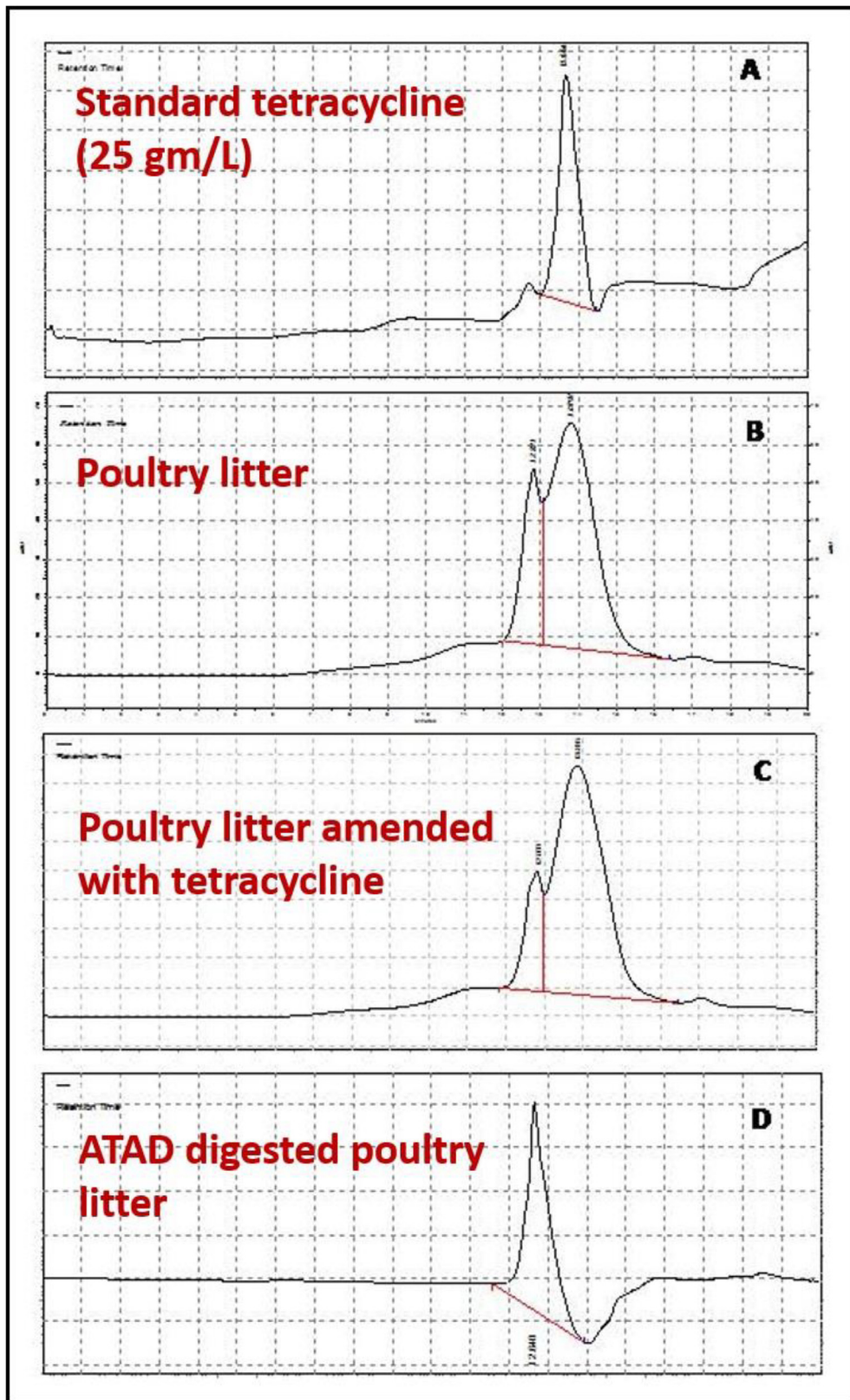


Fig. 2. Typical HPLC chromatograms for (A) Standard tetracycline (25 gm/L), (B) Poultry litter, (C) Poultry litter amended with tetracycline and (D) ATAD digested poultry litter.

Table 1
Validation parameters for tetracycline treated and ATAD digested poultry litter.

Validation parameters	Standard Tetracycline	Poultry litter	Poultry litter with tetracycline	ATAD digested poultry litter
LOD ($\mu\text{g}/\text{kg}$)	5	8	6	9
LOQ ($\mu\text{g}/\text{kg}$)	3	6	4	2
Calibration curve	$Y = 0.001X + 0.066$	$Y = 0.001X + 0.066$	$Y = 0.001X + 0.066$	$Y = 0.001X + 0.066$
Correlation coefficient	0.996	0.996	0.996	0.996
Recovery (%)	96	47	92	0.8

3.2. High performance liquid chromatography

Tetracycline present in poultry litter was detected using HPLC. Tetracycline peak was detected in poultry litter and in poultry litter amended with tetracycline, but not for ATAD digested poultry litter. Interfering peaks were seen in poultry litter, poultry litter amended with tetracycline and ATAD digested poultry litter but it was not seen in the case of standard tetracycline (Fig. 2). This might be due to the existence of compounds in poultry litter which were extracted along with tetracycline. Linearity, precision, exactness, and maximum detection (LOD) were validated.

Linearity was determined through constructing the calibration curve. The maximum detection and the recovery were $5 \mu\text{g}/\text{kg}$ and 96%, respectively for standard TC. Linear correlation curve was obtained over the range of $100\text{--}500 \mu\text{g}/\text{mL}$ with correlation coefficient of 0.996 and calibration curve of $Y = 0.001X + 0.066$. After ATAD digestion, the recovery of tetracycline was 0.8% which was highly reduced when compared with undigested poultry litter containing tetracycline (Table 1).

Antibiotic concentration is reduced due to adsorption or degradation which are temperature dependent. In general, tetracycline has a property to adsorb strongly onto the organic matter which may lead to degradation of the antibiotics (Arikan, 2008). Hartlieb et al. (2003) suggested that during aerobic process (composting) the antibiotic binding sites are generated in the organic matrix where the antibiotic adsorb. Antibiotics excreted from human are eliminated from environment through sorption process (Heise et al., 2006; Kreuzig and Hölting, 2005; Schmidt et al., 2008; Tolls, 2001). Arikan et al. (2006) reported that 60% OTC removal was attained by anaerobic digestion (64 days at 35°C) and this reduction might be due to mineralization, degradation, or binding of OTC to the matrix. Studies have been reported on the reduction of OTC during digestion of soil, sediments and manure as 95%, 80% and 70% at 3, 2 and 4 days, respectively (Arikan et al., 2007; Sarmah et al., 2006; Storteboom et al., 2007). The level of CTC was decreased at the same time the level of 4-epichlortetracycline (ECTC) and isochlortetracycline (ICTC) were increased in outdoor anaerobic pig lagoons, this is because of the sorption of CTC (Arikan, 2008; Meyer, 2005). Dolliver et al. (2008) reported that 99%, 54% and 76% reduction of chlortetracycline, monensin and tylosin were obtained at 1, 17 and 19 days, respectively during composting of manure piles due to changes in manure's physico-chemical characteristics, adsorption or bioavailability reduction. Van Dijk and Keukens (2000) reported that the concentration of sulfachlorpyrazine decreased from 82 to 58% through composting. Storage of this manure for three months showed in an extra reduction (33%) in the concentration of antibiotic.

4. Conclusion

The present study indicates that ATAD can reduce the level of tetracycline in manure to about 96% in 72 h. However, investigations do not decide whether the reduction of tetracycline is through mineralization, degradation or binding of tetracycline to the matrix. Also this may also be due to various factors (raised temperatures, increased biological activity, biologically trans-

formed organic material) which occur in digestion that affect the concentrations of tetracycline but not the reactions among these factors. The effectiveness of ATAD to reduce tetracycline in poultry litter has been evaluated in the present study. It has been proved that ATAD has the potential to reduce antibiotics in poultry litter before the reutilization to reduce environmental health hazards associated to transmission of antibiotic-resistant bacteria to human or other animals.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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