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Evaluation of Degradation of Antibiotic Tetracycline in Pig Manure by Electron Beam Irradiation

Jae-Young Cho

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Abstract This study was carried out to evaluate the degradation efficiency and intermediate products of the tetracycline from artificially contaminated pig manure using of electron beam irradiation as a function of the absorbed dose. The degradation efficiency of tetracycline was 42.77% at 1 kGy, 64.20% at 3 kGy, 77.83% at 5 kGy, and 90.50% at 10 kGy. The initial concentration of tetracycline (300 mg kg^{-1}) in pig manure decreased significantly to 24.2 ± 5.3 mg kg^{-1} after electron beam irradiation at 10 kGy. The radiolytic degradation products of tetracycline were 1,4-benzenedicarboxylic acid, hexadecanoic acid, 9-octadecenamide, 11-octadecenamide, and octadecanoic acid.

Keywords Antibiotic · Electron beam · Radiolysis · Tetracycline

Antibiotics have been instrumental in treating infectious diseases that kill humans and animals. However, their widespread use as an additive in animal feed has raised concerns about the development of antibiotic-resistant microorganisms. A high proportion of the antibiotics added to animal feed is excreted in urine or manure. In some cases, as much as 90% of the antibiotic administered orally may pass through the animal unchanged (Kumar et al. 2005).

In Korea, the sale rate of antibiotics was highest in feed additives. 800 tons of tetracycline was used and it makes up the largest portion among antimicrobials. Direct toxic effects of antibiotics on plants and soil microflora and -fauna are unlikely because they are present at low concentration. However, the indirect effects of antibiotics on the food web cannot be discounted at this stage. Decrease soil microbe populations due to antibiotics in manure may cause loss of food sources for other soil organisms, which, in turn, may affect important soil microbial processes, such as decomposition and mineralization (Kumar et al. 2005).

The degradation of antibiotics and antimicrobials has been investigated in previous studies. For example, degradation of chlortetracycline during the anaerobic digestion of manure (Arikan 2008), ozonation and Fenton process for the treatment of antibiotic containing manure (Uslu and Balcioglu 2009), anaerobic digestion and ozonization technology for treating wastewater from antibiotics production (Qiting and Xiheng 1988), degradation of ampicillin in pig manure slurry and an aqueous solution using electron beam irradiation (Chung et al. 2009a, b), and degradation and inactivation of tetracycline by TiO_2 photocatalysis (Reyes et al. 2006) have been reported.

Among the technologies for the degradation of antibiotics, ionizing radiation has been used to decompose organic substances and environmental pollutants from sewage, sludge, wastewater, and manure and a few papers have included ecotoxicological aspects (Borrely et al. 2000). Within the last decade, several researchers reported that ionizing radiation (electron beam and gamma-ray technology) can transform non-degradable persistent organic pollutants (POPs) to degradable products (Chung et al. 2008, 2009a, b; Kim et al. 2007). However, relatively few studies have reported the usage of electron beam irradiation for the degradation of antibiotics (Chung et al. 2009a, b).

Considering these facts, the efficiency of treatment with electron beam irradiation was evaluated for the degradation

J.-Y. Cho (✉)
Department of Bio-Environmental Chemistry, Chonbuk National University, Jeonju 561-756, Korea
e-mail: soilcosmos@chonbuk.ac.kr

and transformation of tetracycline from artificially contaminated pig manure. This study is the first to investigate the application potential of an integrated process for the destruction of antibiotics in the pig manure.

Materials and Methods

The antibiotic tetracycline was purchased from Sigma–Aldrich (Seoul, Korea). All organic solvents used for sample preparation were residue-analysis grade or better. HPLC-grade solvents were used for liquid chromatography. The stock standard solutions were prepared by dissolving 100 mg of antibiotic in 100 mL of methanol. Standard solutions were stored at −4°C in amber vials and were freshly prepared every 5 months. Working standard solution was prepared in water/acetonitrile (9:1, v/v) by dilution from the stock standard solution. Working standard solutions were used for fortification in recovery experiments and for preparation of calibration standards.

Pig manure was collected from farm located at Iksan-si, Jeollabuk-do, Republic of Korea. After drying at 60°C for 24 h, manure was passed through a sieve (2 mm). It was sterilized in an autoclave at 120°C for 10 min to prevent biological activity and then stored at 4°C until use. Each manure sample was contaminated by spiking it with the tetracycline stock solution that was prepared in water at a concentration of 300 mg L^{-1}. After mixing thoroughly with a vortex mixer, the contaminated manure was allowed to equilibrate for 2 h (Uslu and Balcioglu 2009). Electron beam irradiation was performed using an EB-TECH unit (ELV-4, 1.0 meV, 40 KW) at the Advanced Radiation Technology Institute located in Jeongeup-si, Jeollabuk-do, Republic of Korea. The samples were placed in polyethylene film bags and radiation exposure was performed at doses of 1, 3, 5, and 10 kGy. After irradiation, all samples were stored at 4°C until analysis (Chung et al. 2009a, b).

Bautitz and Nogueira (2007) have previously described the details concerning sample preparation, standard calibration, recovery testing and the analysis of tetracycline using HPLC and LC-MS system. Briefly, the samples were extracted using solid phase extraction Oasis cartridges. A solution of 0.01 mol L^{-1} oxalic acid in methanol was used for conditioning the cartridges and elution of the sample. After solid phase extraction, tetracycline analysis was performed using HPLC (Waters 2690). The analytical conditions were as follows; detector: Waters 996 photodiode array detector; column: C_{18} reverse phase column (5 μm, 4.6 × 250 mm); flow rate: 1 mL/min; detector wavelength (λ): 355 nm; injection volume: 50 μL. The mobile phase used as eluent was 0.01 mol L^{-1} oxalic acid:methanol:acetonitrile (72:8:20). Recovery tests for tetracycline were carried out at three concentration levels (5, 10 and 15 mg L^{-1}), resulting in an average recovery of 84.4% with a 4.3 coefficient of variation (n = 3) (Chung et al. 2009a, b).

Results and Discussion

Electron beam irradiation of aqueous solutions generates various radicals by water radiolysis according to the following reaction:

$$H_2O \rightarrow [2.7]HO^{\bullet} + [2.6]\ e^{-}_{aq} + [0.6]H^{\bullet} + [0.7]H_2O_2 + [2.6]H_3O^{+} + [0.45]H_2$$

Values in brackets (G) are the radiation chemical yields, i.e., the number of species formed in water absorbing energy of 100 eV. In acidic conditions, aqueous electrons react with hydronium ions to form H· radicals (Schwartz 1981):

$$e^{-}_{aq} + H_3O^{+} \leftrightarrow H^{\bullet} + H_2O\ K = 3.98 \times 10^{9}$$

According to these reactions, hydroxyl radicals and hydrogen atoms are the major species (Unob et al. 2003).

The pig manure samples were irradiated at 1, 3, 5, and 10 kGy with an electron beam in the presence of air. The spiked initial concentration of tetracycline in pig manure was 300 mg kg^{-1}. Results were expressed as C/C_0; where C_0 and C represent the concentration of tetracycline before and after electron beam irradiation, respectively.

The chromatogram and degradation efficiency of tetracycline in pig manure at various electron beam doses are shown in Fig. 1. The degradation efficiency of tetracycline was 42.77% at 1 kGy, 64.20% at 3 kGy, 77.83% at 5 kGy, and 90.50% at 10 kGy. The initial concentration of tetracycline (300 mg kg^{-1}) in pig manure decreased significantly to 24.2 ± 5.3 mg kg^{-1} at 10 kGy after electron

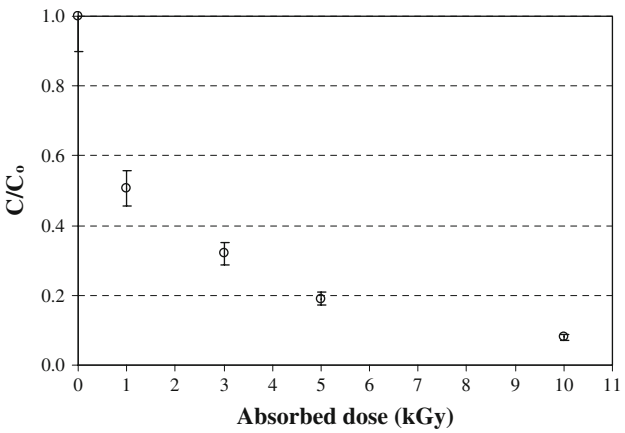

Fig. 1 Degradation of tetracycline in pig manure at after electron beam irradiation a various doses

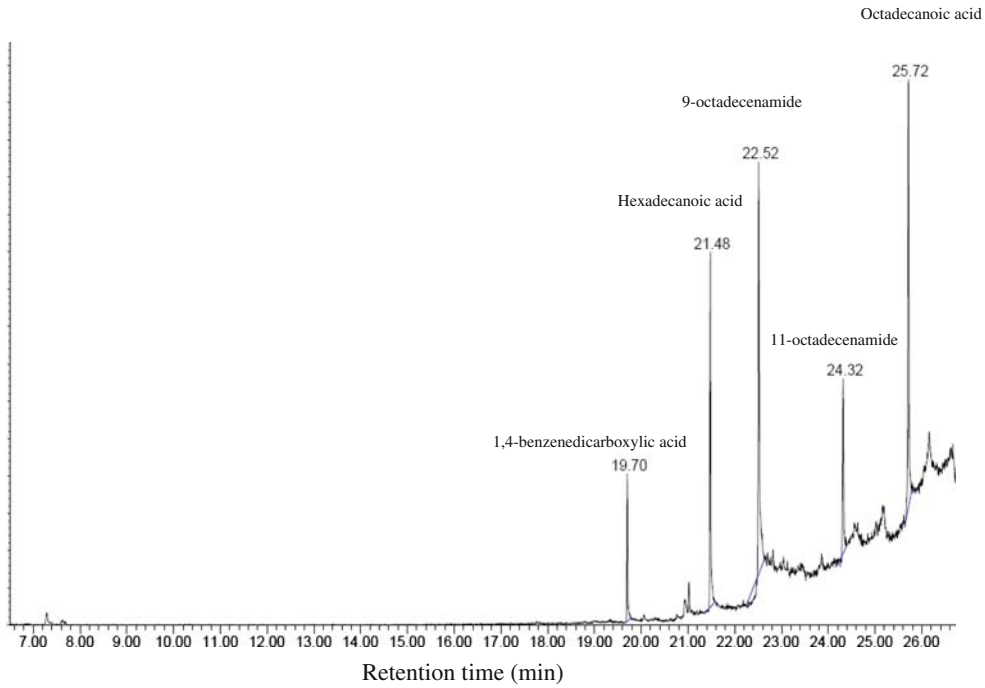

Fig. 2 Intermediate products of radiolytic degradation of tetracycline

beam irradiation. An HPLC/MS method was applied using standard solutions of tetracycline to confirm degradation products. The retention times (t_R) of peaks and the chemical structure of identified substances are shown in Fig. 2. Radiolytic degradation products of tetracycline were 1,4-benzene dicarboxylic acid (t_R: 19.70), hexadecanoic acid (t_R: 21.48), 9-octadecenamide (t_R: 22.52), 11-octadecenamide (t_R: 24.32), and octadecanoic acid (t_R: 25.72). Based on these products, we conclude that the radiolytic degradation of tetracycline involved the opening of the aromatic ring and the oxidation of aliphatic acids.

In our previous work (Chung et al. 2009a, b), in the tetracycline aqueous solution, the degradation efficiency was 83.5% at 1 kGy, 91.5% at 3 kGy, and 100.0% at 5 and 10 kGy. The radiolytic degradation products of tetracycline were 1,4-benzenedicarboxylic acid, Hexadecanoic acid, and 9-octadecenamide. Our present results are in general agreement with our previous work with minor differences that may be due to the different matrix (pig manure and aqueous solution). This could be due to several organic materials in pig manure used in the present study; further studies are necessary. The present study demonstrated that electron beam irradiation technology is an effective means of removing antibiotics from manure and livestock wastewater.

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