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Sorption and Desorption Characteristics of Tylosin in Three Louisiana Soils and Clay Minerals

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SORPTION AND DESORPTION CHARACTERISTICS OF TYLOSIN IN
THREE LOUISIANA SOILS AND CLAY MINERALS

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ABSTRACT

Tylosin, a macrolide class and widely used antibiotic in animal production, could contaminate environment through manure application to soil and further to river and ground water. Tylosin transport and movement in the environment are largely determined by its sorption and desorption in soils, which are poorly understood so far. Therefore, the purpose of this study was to determine (1) the optimal conditions for tylosin stability, (2) sorption and desorption characteristics of tylosin by poultry litter-impacted three Louisiana soils and (3) sorption and desorption of tylosin by selected clay minerals, the main components of soil which have strong potential application in environmental clean-up. To this end, tylosin stability was evaluated in terms of light exposure (light and dark), solvents (H_2O and 0.01M $CaCl_2$), pH (4.5 to 7.5), and temperature (4 ^{o}C and 25 ^{o}C) conditions. Sorption and desorption of tylosin were carried out at different pHs with three Louisiana soils namely Briley, Ruston and Savannah with or without organic matter being removed. For clay minerals, tylosin sorption and desorption were carried out with montmorillonite, kaolinite and illite. Tylosin in 0.01M $CaCl_2$ was stable under light at pH 4.5, 6.0 and 7.5, and at 25 ^{o}C for about 12 days. Tylosin sorption was well described by the Freundlich equation, with sorption in the order of Briley > Savannah > Ruston. Tylosin sorption was affected by pH, with higher sorption at acid pH for Briley, and at pH 6 to 7 for Ruston and Savannah, but with low sorption as pH further increased. Removal of organic matter dramatically increased tylosin sorption and changed sorption pattern at lower pH < 6. Tylosin desorption was in the order of Ruston > Briley > Savannah, with higher desorption under acid conditions, and lower desorption between pH 6.0 to 7.5. Tylosin was strongly sorbed by monotmorillonite followed by illite and kaolinite, and desorption in the order of illite > kaolinite > montmorillonite. The results indicated that both pH and organic matter significantly affected

tylosin sorption and desorption behaviors in soil and three clay minerals exhibited different

degree of sorption strength as reflected by the difference in desorption.

CHAPTER 1

LITERATURE REVIEW

1.1 Introduction

Antibiotics are a diverse range of compounds produced naturally by various living organisms, such as bacteria, yeasts, fungi or manufactured synthetically (Kanfer et al., 1998). They have antibacterial activity which can inhibit or destroy certain organisms, such as bacteria, and are widely used for disease treatments, prevention, and control, as well as growth promotion. The use of antibiotics has become an integral part of the growing animal food production industry. To maintain animal health, large amount of antibiotics are consumed. Although there is no official report of antibiotics usage, a survey by Animal Health Institute (AHI) showed that about 9 million kg of antibiotics were sold by AHI members in 2001 (AHI, 2002). It was estimated that > 22 million pounds of antibiotics were used to treat farm animals and pets in United States in 2002 (Kolpin et al., 2002). Majority of antibiotics used in U.S. are for preventing and curing illness, with only 13% for growth promotion. According to a review by Sarmah et al. (2006), 5 million kg of antibiotics were used in 2000 in European countries for animal health, among which 3.5 million kg were used for therapeutic purposes, and 1.5 million kg were used as food additive for growth promotion. In New Zealand, antibiotics are used in feed because of large population of ruminant animals on pasture, animals consumed about 57% of nearly 93,000 kg of antibiotics. In Kenya, 14,600 kg of antibiotics were used in animal food production.

The use of antibiotics in animal food production has been a concern for the public, and controversial to the scientific community because of potential environmental contamination, including water and land. Many antibiotics used in animal production are poorly adsorbed by

animals, and as much as 30-90% of the parent compound is excreted (Alcock et al., 1999). In addition, metabolites of antibiotics can also be bioactive and some are transformed back to the parent compound after excretion. For example, the excreted sulfamethazine metabolite, glucoronide of N-4-acetylated sulfamethazine, is converted back to the parent compound in liquid manure (Berger et al., 1986). After the antibiotic is administered, sulfamethazine undergoes conjugation with sugars in the liver and thus becomes inactivated. After excretion, sugar is rapidly degraded by microbes, thereby reconverting the conjugate to bioactive form. So, it is not surprising that nearly 21% of an oral dose of oxytetracycline was excreted from sheep as the original parent compound, and 17-75% of chlortetracycline was excreted from young bulls (Montforts, et al., 1999). Moreover, most of the antibiotics are water-soluble, as much as 90% of an antibiotic dose can be excreted in urine and up to 75% in animal feces (Halling-Sorensen, 2001). Consequently, antibiotics are frequently found in feces, urine, slurry, manure, and dung (Cole et al., 2000; Halling-Sorensen et al., 2002) as manure, slurry and dung are widely applied as fertilizers to land. It is therefore very likely that antibiotics, released into soil through fertilization, could find their way into streams and rivers through runoff, resulting in water contamination. For example, a nationwide survey in the U.S. revealed that numerous veterinary and human antibiotics were detected in 27% of 139 river water samples at concentrations of up to 0.7 μg L^{-1} (Kolpin et al., 2002). Antibiotics such as tetracycline and sulfonamide were found in water of the Colorado Cache la Pouder River flowing through urban and agricultural areas (Yang et al., 2003). In addition to surface water, antibiotics were also found in ground water from urban and agricultural sources (Sengelov et al., 2003; Richards et al., 2004). These results suggest that antibiotics pose an increasingly urgent risk to the environment.

Antibiotics have been also reported in plant-based food such as grain, vegetable and fruit through uptake by roots. As early as the 1950s, antibiotics were found in the tissues of broad-

bean plants grown in solutions containing antibiotics griseofulvin and chloramphenicol (Crowdy et al., 1955). In recent studies, antibiotics such as chlortetracycline were detected in corn, green onion and cabbage grown in soils applied with manure containing chlortetracycline (Kumar et al., 2005). Another antibiotic compound, sulfamethazine, was also found in corn, lettuce and potato grown in manure-amended soil (Dolliver et al., 2007). Obviously, there is also a potential risk of antibiotics directly to human for consumption of these food contaminated with manure application.

The main concern over frequent use of antibiotics in animal production has been the potential for an increase in populations of new strains of microorganisms resistant to antibiotics (Witte, 1998). Bacterial populations isolated from the gut of animals exposed to antibiotics were five times more likely to be resistant to those antibiotics, and tetracycline-resistant bacteria were identified in manure (Halling-Sorensen et al., 2005). Antibiotics sorbed on Minnesota soils maintained their antibacterial activity, thus putting continuous pressure on bacteria (Chander et al., 2005). Under the pressure of antibiotics, sensitive strains could acquire resistance either from gene mutation or from exchromasomal exchange of resistant genes from R plasmids to become resistant strains. Similarly, antibiotics remaining in soils may favor development of antibiotic-resistant bacteria (Halling-Sorensen et al., 1998). A small number of resistant bacteria will gradually become major strains under continuous selection pressure. Using polymerase chain reaction (PCR) technique, bacteria with resistance to tetracycline and streptomycin have been found one year after their exposure to antibiotic (Sengelov et al., 2003), indicating that the antibiotic-resistant strains develop much faster than expected though initially they are not dominant in the populations. Consequently, bacteria in soils become more resistant to antibiotics (Esiobu et al., 2002), and tylosin-resistant bacteria have been isolated from a Minnesota cornfield to which swine and chicken manure historically had been applied (Onan et al., 2003).

These antibiotic-resistant bacteria could spread into water from soils through runoff, and causes serious water contamination. Once these antibiotic-resistant bacteria spread into animals or humans and become dominant in populations, relevant antibiotics would lose their protective activities for animals and humans.

Based on chemical structure, antibiotics used for veterinary medicine are classed into tetracyclines, aminoglycosides, macrolides, β-lactams, sulfonamides, and fluorquinolones (Thiele-Bruhn, 2003). As one member of macrolides, tylosin is one of the most widely used veterinary antibiotics in the United States, European Union (EU), Australian, New Zealand and several other countries. It has been administered to swine, beef cattle and chicken for disease prevention and control as well as growth promotion. The amount of tylosin administered in animals is high. For example, Denmark used 14,000 kg of tylosin in 1997 and UK 5144 kg of tylosin in 2000 (Loke et al., 2000; Smarmah et al., 2006). Due to the fact that veterinary antibiotics likely enter the environment through manure application to agricultural lands, sorption and desorption behaviors of antibiotics in soils will likely play a major role in controlling mobility and fate of these antibiotics. Therefore, tylosin sorption and desorption characteristics in soils and influencing factors will be focus of this study. In addition, the interaction between tylosin and specific clay minerals will be also studied.

1.2 Literature review

1.2.1 General Properties of Tylosin

Tylosin, a member of macrolide group of antibiotics, has good antibacterial activity against a broad-spectrum of pathogenic organisms such as gram-positive bacteria, some gram-negative bacteria, vibrio, spirochete, coccidian, etc. (McGuire et al., 1961). Tylosin is produced by fermentation of *Streptomyces* strains. Structurally, it consists of a substituted 16-membered lactone ring, an amino sugar (mycaminose), and two neutral sugars (mycinose and mycarose).

Like other fermentation products, tylosin is a mixture of the tylosin A (macrolide), tylosin B (desmycosin), tylosin C (macrocin), and tylosin D (relomycin). Their chemical structure is listed in Fig. 1.1 (Loke et al., 2000).

All four compounds contribute to the potency of tylosin. Among these four compounds, tylosin A is the major one, accounting for 80% to 90%, followed by tylosin B (Horie et al., 1998). Tylosin A, tylosin B, tylosin C and tylosin D were found in faeces, suggesting that tylosin B, tylosin C and tylosin D are the metabolites of tylosin A (Horie, 1995).

Tylosin, a weak organic base with pK_a of 7.73, is not stable in acidic and alkaline conditions. In acidic conditions, tylosin A is converted to tylosin B, whereas in neutral and alkaline condition it may decompose to tylosin A aldol along with a number of polar
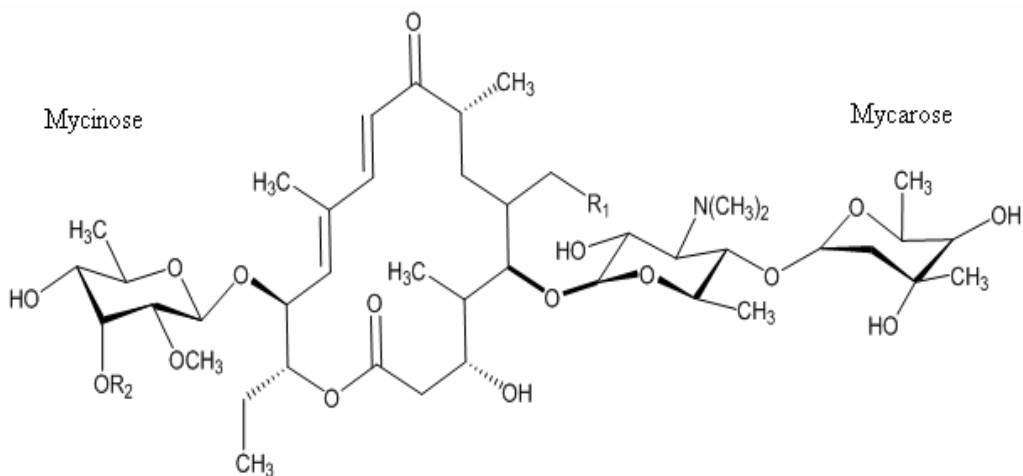
Substance	R_1	R_2	Mycarose	Mycinose
Tylosin A	CHO	CH$_3$	+	+
Tylosin B	CHO	CH$_3$	-	+
Tylosin C	CHO	H	+	+
Tylosin D	CH2OH	CH$_3$	+	+

"+" sugar present; "-" sugar not present.

Fig. 1.1 Chemical structure of tylosin

decomposition products (Paesen et al., 1995). However, tylosin remains relatively stable at pH around 7 in aqueous solution.

Like many other antibiotics, tylosin is subject to photodegradation. Although tylosin concentration remained stable in the dark, when exposed to light, it degraded by 13% (Halling-Sorensen et al., 2003). Tylosin is widely used in animal feed for pig, cattle and poultry production for the treatment of diseases and growth promotion.

1.2.2 Occurrence of Tylosin in Environment

Tylosin has been used in animal feeds and for animal disease control for approximately 40 years. According to a report by Hu (2007), after administering, up to 90% of drugs can be excreted in feces and urine as metabolites or in the parent form. Tylosin was the most frequently used antibiotic at 31% of swine production facilities (Bush et al., 2001). Through application of manure with excreted tylosin residues from livestock on croplands, without question tylosin can enter into soil and water system as a pollutant in the environment. Different studies have reported that the half life of tylosin ranged from 2 to 200 days in different matrics (Table 1.1), so that tylosin residue might accumulate in our environment following manure application. Recent evidence has indicated that tylosin has been indeed released into the environment. According to Koppin et,al. (2002), tylosin was detected in 14% of 139 streams in the United State in 2002 (Kolpin, et al., 2002). As much as 0.012 μg L^{-1} of tylosin was found in Arkansas streams along with other antibiotic compounds (Haggard et al, 2006). Clearly, as the occurrence of tylosin in the environment is beginning to be understood, further understanding of its fate and strategy can be developed in managing the risk associated with tylosin contamination.

1.2.3 Tylosin Sorption to Soils, Clay Minerals and Manure

Previous studies suggested that tylosin is strongly sorbed in soils, but sorption as determined by sorption partition coefficient (K_d) varied greatly. The k_d for 11 different Dutch

field soils ranged from 10 to 370 (Laak et al., 2006). Tylosin sorption appeared to correlate

positively with the soil physical properties. A study by Rabolle et al. (2000) found that K_d vales

for a sandy and two Danish sandy loam soils were 8-11, and 62-128, respectively, suggesting

that soil with higher percentages of silt and clay had higher sorption of tylosin. This was

supported by Clay et al.'s (2005), who found that Kranzburg soil from South Dakoda, with

63.3% silt, 29.1% clay and 7.6% sand, exhibited higher adsorption of tylosin than Badger soil,

which had 56.6% silt, 31.0% clay and 12.4% sand. Similarly, a Dutch clay loam soil sorbed

more tylosin than a Dutch loamy sand soil (Laak, et al., 2006), and a Canada Quebec heavy clay

soil sorbed more tylosin than a Canada sandy loam soil (Allaire et al., 2006). Tylosin sorption is

rapid, with 95% of it sorbed within 3 h (Allairie et al., 2006).

Besides soil physical properties, soil pH and ionic strength can also affect tylosin sorption.

Tylosin is a weak base, and pH may change the charge characteristics of both tylosin and soil

particles, thus affecting sorption. A study with Dutch clay loam soil and loamy sand soil showed

that sorption of tylosin on soil was higher under weak acid conditions (pH 6). As pH increased,

the sorption partition coefficient decreased from 156 to 32 for the clay loam soil and from 8.9 to

3.0 for loamy sand soil (Laak, et al., 2006). Addition of an electrolyte ($CaCl_2$ or NaCl) at more

than 0.03 M led to decrease sorption of tylosin by 3-fold (Laak, et al., 2006).

Similar to sorption by soil, tylosin is also sorbed by manure. However, sorption of tylosin

on solid manure was affected by solvents, with sorption from urine three to four times greater

than with sorption from 0.01 M $CaCl_2$ and water (Clay et al., 2005).

Clay minerals are the sorbent in soil, but it varys considerably in adsorption for tylosin.

In an early study by Bewick (1979), Na-montmorillonite and Ca montmorillonite (Wyoming

bentonite) clay minerals were found to exhibit high adsorption of 190 and 65 $\mu g \ mg^{-1}$

Table 1.1 Tylosin half life in different environmental matrices

Half-life (T1/2 = day)	Matrix	Reference
4.1-8.1	Soil-manure slurries in aerobic laboratory	Halling-Serensen (2001) Ingerslev (2001)
49-67 and 79-82	Nature soil	Halling-Serensen (2005)
4.5	Manure	Carlson (2006)
< 2	Aqueous phase in manure	Loke et al.(2000)
9.5-40	Surface water	Ingerslev (2001)
6	Cattle excreta	Teeter and Meyerhoff (2003)
< 7.6	Chicken excreta	
7.6	Swine excreta	
200	Pond water and ultra water in light	Hu et al. (2007)

respectively, whereas illite and kaolinite had lower adsorption of 22 and 6.5 μg mg^{-1} respectively. The difference was attributed to expand the structural variation of the clay minerals. Both bentonite and montmorillonite have an expanding lattice that results in an increased exchange capacity as compared to illite and kaolinite, which have no expanding lattice.

1.2.4 Tylosin Desorption from Soils

Two reported studies have been conducted on desorption of tylosin from soils, but the results were quite different. A study by Clay et al. (2005) found that desorption of tylosin from South Dakota soil was low. In a 24-h period, tylosin desorption with 0.01 M CaCl$_2$ from three soils was less than 0.2% of the total tylosin sorbed at a high concentration. No tylosin was desorbed from soils exposed to a low tylosin concentration. However, an earlier study conducted by Rabolle et al. (2000) indicated much higher desorption. Using similar desorption methods, Rabolle et al (2000) found that 69% and 26% of sorbed tylosin were desorbed from Danish loamy Borris and Lunggaard soils, respectively.   In sandy loam soils, desorption was lower to 13-14% of the sorbed tylosin. These inconsistent results suggest that the factors influencing tylosin desorption in soils are not clear.

1.2.5 Tylosin Mobility and Fate in Soils

Due to its high sorption onto soil, tylosin mobility in soil is limited. A study using soil column showed that tylosin moved slowly in soils, but differences existed among soils (Rabolle et al., 2000). In a Danish sandy loam soil, almost all the tylosin remained in the top 0-2.5cm layer of soil, with very small amounts transported to the 2.5-5.0 cm and 5.0-7.5 cm depths. In contrast, tylosin moved to 20 cm in a sandy soil, with small amounts detected in the 20-22.5 cm and 22.5-25 cm depths (Rabolle et al., 2000). There has been no report on tylosin movement in runoff.

There are few studies on the fate of tylosin in soil. Ingerslev and Halling-Sorensen (2001) simulated the biodegradability of three veterinary antibiotics in Danish soil-manure slurries under aerobic laboratory conditions using aniline as the benchmark chemical, and found that half-life for tylosin was 4.1-8.1 days. In soil situations where soil pH, redox, temperature, water content, composition and microbial population vary considerably, the fate of tylosin may be quite different. Halling-Sorensen (2005) examined the fate of tylosin in natural soil conditions during a 155-day experiment with two Danish soil types. Degradation of tylosin not only depended on subtype of tylosin, but also on soil. Tylosin A degraded faster than tylosin B and tylosin D. The half-life of tylosin A was 49-67 days in sandy to loamy sand soils, whereas that of tylosin B and of tylosin D was 84-114 days and 79-82 days respectively in similar soils. The content of tylosin C remained stable, showing little sign of degradation. In addition, it is generally believed that tylosin B, tylosin C and tylosin D are the degradation products of tylosin A, and as compared to parent compound tylosin A, these were more persistent in the soil (Halling-Sorensen, 2005). Another study by Carlson et al. (2006) indicated that the half-life for tylosin was 6.1 days in Ontario soils, and addition of manure in these soils seemed to reduce it to 4.5 days.

Both aerobic degradation and abiotic degradation were found to be involved in tylosin degradation in soils (Sassman et al., 2007). Between the non-sterile and the sterile Florida soils, tylosin degradation was similar during the first two weeks, suggesting that abiotic degradation may dominate at early times. Aerobic degradation appeared to be more rapid than anaerobic, with more than half of the tylosin degraded within 3 days after a long lag time (Sassman et al., 2007).

1.2.6 Tylosin Stability in Water, Sewage and Manure

Under aerobic conditions, tylosin was relatively stable in surface water. Placed in a simple shake flask system simulating the conditions in surface water, tylosin had a half-life of 9.5 to 40 days (Ingerslev et al., 2001). Addition of sediment or sludge increased its degradation, however, degradation was significantly slower in the absence of oxygen.

Tylosin potencies were affected by soil interstitial water and sludge water. Soil interstitial water reduced the potency of tylosin within two days under anaerobic condition, with EC_{50} (50% effective concentration) increased from 2.5 to 15.9 mg/L, but tylosin concentration remained constant (Halling-Sorensen et al., 2003). The potency of tylosin in sludge water was reduced by 28.2% in 10-h experimental period, but the initial concentration of tylosin was only reduced by 2.2%. This behavior was due to conversion of tylosin into other less potent forms such as tylosin B, tylosin C, tylosin D (Teeter et al., 2003). In addition, biodegradation, sorption to sludge, or combinations of the both processes may also reduce the potencies of tylosin in sewage sludge (Teeter et al., 2003).

Tylosin degraded rapidly in manure. One study (Liguoro et al., 2003) showed that as much as 115,500 µg/kg tylosin was excreted from calves into manure after feeding tylosin at a rate 20 mg/kg/day, but this amount was greatly degraded to 5,400 µg/kg in the manure by day 10. No tylosin was detected in the manure after 45 day (Liguoro et al., 2003). Mixing tylosin with manure also resulted in its degradation. In an aqueous solution of containing 0.1% manure, no tylosin degradation was detected in neither manure filtered through a 0.45 µm filter (no microbes) nor non-filtered manure. With an increase of manure to 3%, degradation rate was increased dramatically in non-filtered manure. In contrast, degradation rate was lower in filtered manure (Loke et al., 2000). This suggested that sorption of tylosin to manure particles, or/and

microbe degradation contributed to lower tylosin content in non-filtered manure solution. Using a ^{14}C-tylosin spiking solution, the half-lives of tylosin in cattle, chicken and swine excreta were estimated to be 6.2 days, < 7.6 days and 7.6 days, respectively (Teeter et al., 2003).

1.3 Statement of Problems and Objectives

Widespread application of animal waste to agricultural land has caused concern about the introduction of veterinary antibiotics into the environment. One such commonly used antibiotic for poultry and cattle production is tylosin. However, there is little information on the mobility and fate of tylosin in soils.   The interaction of tylosin with soil components is a key to understanding its retention and release in soil. Therefore, the objectives of this study were to (1) evaluate the stability of tylosin under different conditions of light exposure, temperature, and pH; (2) characterize tylosin sorption, desorption, and factors that influence these in selected Louisiana soils; and (3) assess tylosin sorption and desorption by clay minerals.

This information is critical for assessing the environmental risks associated with tylosin and developing the necessary means to manage these risks**.**

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CHAPTER 2

TYLOSIN STABILITY UNDER DIFFERENT CONDITIONS

2.1 Introduction

Tylosin is one of the most widely used antibiotics for disease control, disease prevention and growth promotion in animal production. Its structure was shown in chapter 1 (Fig. 1.1). The amount of tylosin administered in animals is high. For example, Denmark used 14,000 kg of tylosin in 1997 and UK 5144 kg of tylosin in 2000 (Loke et al., 2000; Smarmah et al., 2006). Tylosin is produced by fermentation of *Streptomyces* strain. Structurally, it consists of a substituted 16-membered lactone ring, an amino sugar (mycaminose), and two neutral sugars (mycinose and mycarose). Tylosin is a mixture of tylosin A, tylosin B, tylosin C and tylosin D (See Fig 1.1). All four components contribute to the potency of tylosin. Among these four components, tylosin A is a major one accounting for 80% to 90%, followed by tylosin B (Horie et al., 1998). Chemically tylosin is a weak base with pKa 7.73.

Tylosin stability is not well documented in the literature. Among the factors affecting tylosin stability, solution pH is thought to be the most important one. It is generally considered that tylosin is stable in neutral pH condition, but not in acidic and alkaline conditions (Paesen et al., 1995a). In acidic conditions, tylosin is converted into tylosin B, whereas in neutral and alkaline conditions it decomposes to tylosin A aldol along with a number of polar decomposition products (Paesen et al., 1995a). Below pH 4, the sugar moieties are cleaved from the parent molecule. Above pH 9, loss of sugar as well as condensation at the R1 aldehyde group gives the aldols. Another factor affecting tylosin stability is light exposure. One study showed that tylosin concentration remained stable in the dark. But when exposed to light, it decreased by 13 % (Halling-Sorensen et al., 2003), indicating photodegradation.

To study tylosin sorption in soils or other media, one fundamental requirement is to conduct experiments under certain conditions, in which tylosin is stable, so only sorption contributing to tylosin removal in equilibrating solutions can be derived. Thus, tylosin stability was evaluated in this study under different conditions of light exposure, solvents, pHs and temperatures to determine the optimal conditions for tylosin sorption.

2.2 Materials and Methods

Tylosin as tylosin tartrate was purchased from Sigma-Aldrich, Inc (St. Louis, MO, USA). Selected physical and chemical properties of tylosin are given in table 2.1. Methanol (HPLC grade), sodium perchloride (HPLC grade), and acetonitrile (HPLC grade) were obtained from Fisher scientific Inc (Pittsburgh, PA, USA).

Table 2.1 The selected properties of tylosin

Molecular formula	$C_{46}H_{77}NO_{17}$
Appearance	A white or slightly yellow crystalline power
Molecular weight	916.1
Solubility	5 mg mL^{-1} (water 25 ^{o}C)
Stability	Solution are stable at pH 4-9
pKa	7.73
UV absorbance	282nm

(Paesen et al.,1995b and c; McFarland et al.,1997)

● **Effect of Light and Air Exposure on Tylosin Stability**: Tylosin was dissolved in sterile H_2O at a concentration of 10 mg L^{-1} and kept at 25 ^{o}C in clear glass vials in the dark with cap closed (dark-close), in the light with cap closed (light-close), and in the light with cap opened (light-open) for 0, 2, 4, 6, 8, 10, 12, 14, 19 and 24 days. A 2-mL of tylosin solution was collected at each time interval for quantification by high performance liquid chromatography

(HPLC). The light-open vials were weighed every day to determined loss of volume due to evaporation. The tylosin concentration was corrected for evaporation.

● **Calcium Chloride and pH Effect on Tylosin Stability:** Tylosin was dissolved into H_2O and 0.01 M $CaCl_2$ with pH pre-adjusted to 4.5, 6.0 or 7.5 to yield final concentration of 10, 25, and 50 mg L^{-1}, respectively. Tylosin solutions were then kept in the dark. An aliquot was taken at 0, 1, 4, 7, 12, 18 and 25 days from each solution for tylosin quantification by HPLC. In addition, tylosin stability in H_2O and 0.01 M $CaCl_2$ solution without pH adjustment for 27 days was evaluated. The half-life of tylosin in H_2O and 0.01 M $CaCl_2$ at pH 4.5, 6.0 or 7.5 was estimated by using the first-order kinetic model, which can be expressed as

$$Ln\ C_t = ln\ C_0 - kt$$

Where C_0 and C_t (mg L^{-1}) are the concentrations of tylosin at time 0 and t (day) and k is the rate constant (Sparks 2003). Half life was the time (t) when C_t is equal to 1/2 C_0.

● **Temperature Effect on Tylosin Stability:** Tylosin, dissolved in 0.01 M $CaCl_2$ solution at a concentration of 10 mg L^{-1}, was kept at 4 ^{o}C and 25 ^{o}C for 0, 1, 4, 7, 12, 18 and 25 days. An aliquot was taken at each time interval and analyzed for tylosin concentration by HPLC. All above experiments were conducted in duplicate.

Tylosin in the solution was analyzed using an Agilent 1100 HPLC system (Agilent Technologies, Palo Alt, CA, USA), equipped with ODS column (250 x 460 mm i.d.) and 5 μm particles. The mobile phase consisted of sodium perchlorate (2.25% m/v) adjusted to pH 2.5 with hydrochloric acid, and acetonitrile (60:40 v/v). The flow rate was set at 1.0 ml/min, and the column was operated at 35 ^{o}C. The injection volume was 25μl. The detection was carried out at a wavelength of 290 nm. A series of standards were prepared and analyzed with samples in the same HPLC run. A typical HPLC chromatography is shown in Fig. 2.1 and typical standard curve is shown in Fig. 2.2.
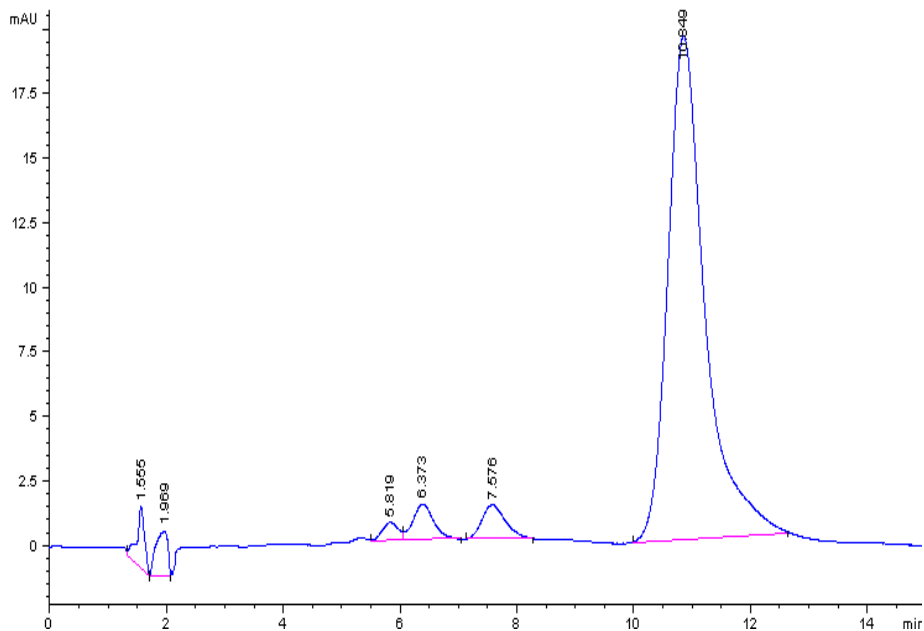
Fig. 2.1 A typical HPLC chromatography of tylosin in H_2O. Tylosin A absorbance peak appeared at about 10.8 min.
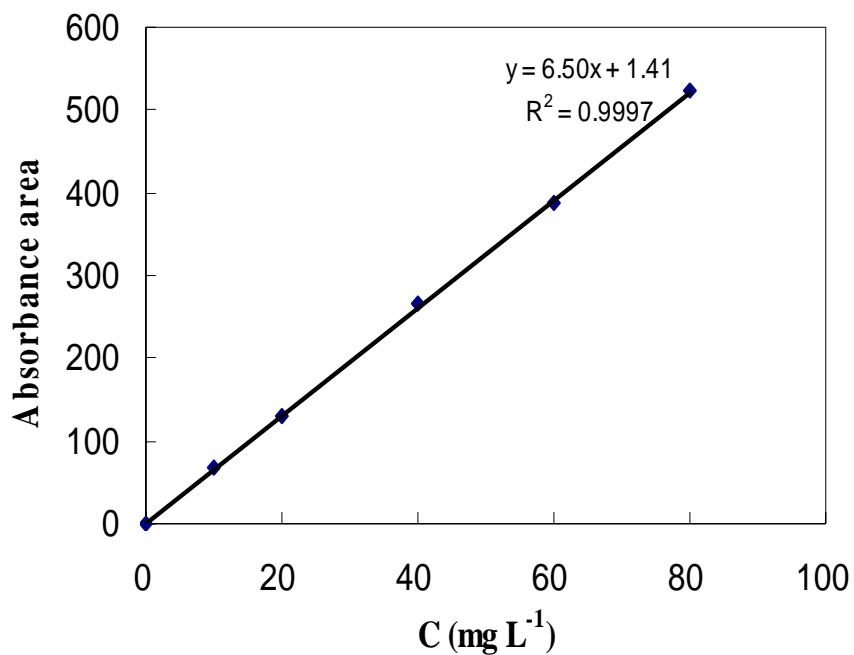
Fig. 2.2. A standard curve of absorbance area versus tylosin concentration (mg L^{-1}). Absorbance areas were highly correlated with amount of tylosin in a linear equation, with R^2 close to 1.

2.3 Results and Discussion

2.3.1 Tylosin Stability Under Dark and Light Conditions

The effect of light and air exposure on tylosin stability in H_2O is shown in Fig. 2.3. In general, the concentration of tylosin was similar under dark-close and light-close conditions within 12 days, suggesting that there was no photodegradation during this short period. There was a slight reduction in tylosin concnetration over 12 days, but it was still similar under both light and dark conditions, showing no sign of degradation within experimental period. It was reported that half-life of tylosin was as long as 200 days under 12 h light daily condition (Hu and Coats, 2007), so it is reasonable that tylosin is stable in a short period as 12 days. But tylosin is
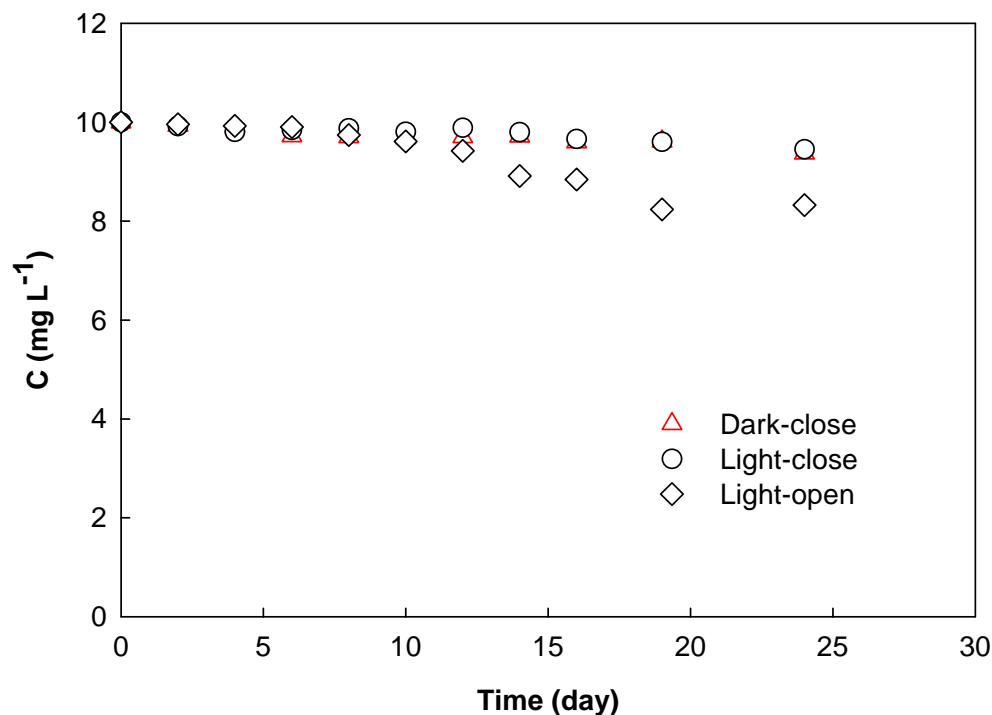
Fig. 2.3. Tylosin stability under dark and light conditions in H_2O. Tylosin solutions (10mg L^{-1}) were incubated in dark with vial cap closed (dark-close), light with vial cap closed (light-close) and light with vial cap opened (light-open) for times shown at 25 ^oC.

slowly degraded over longer time.   Light appeared to expedite degradation. According to Hu's study, 50% of tylosin was lost under 12-h light exposure in 200 day but only 6% tylosin was lost

in the dark over a period of 6 months (Hu and Coats, 2006). Our study is in agreement with this study in that tylosin is relatively stable in water. As most tylosin sorption/desorption studies were conducted in a shorter period, it is not necessary to apply light prevention measure.

Significant degradation, however, occurred after 10 days of exposure to light when vial cap was opened (Fig.2.3). This was likely due to microbial contamination that resulted in biotic degradation. It was found that tylosin degradation in sterile water was much slower than in surface water manure (Ingerslev et al., 2001), and biotic degradation was responsible for tylosin degradation (Loke et al., 2000). This suggests that use of sterile water is important in studies involving tylosin stability.

2.3.2   Tylosin Stability in H_2O and 0.01 M $CaCl_2$ and pH Effect

Ionic strength is one factor affecting tylosin stability. Studies have shown that degradation rate increased with background ionic strength (Pasen et al., 1995a). Adsorption of tylosin to soil also increased with $CaCl_2$ concentration (Laak et al., 2006). The use of 0.01 M $CaCl_2$ in solution studies was primarily for mimicking soil ionic strength with the dominance of Ca ions (Sparks, 2004). However, there was no report about tylosin stability in 0.01 M $CaCl_2$. Because 0.01 M $CaCl_2$ was widely used to dissolve tylosin for studying the behavior of tylosin in soil, tylosin stability in 0.01M $CaCl_2$ was compared with that in H_2O in this study. To that end, tylosin was placed in 0.01M $CaCl_2$ or H_2O at concentrations of 10, 25 and 50 mg L^{-1} over a period of 27day. The results in Fig. 2.4 showed the tylosin stability for an initial concentration of 10mg L^{-1}. There was no significant difference in tylosin concentrations between 0.01 M $CaCl_2$ and H_2O over 27 days of incubation, suggesting that 0.01 M $CaCl_2$ did not cause tylosin degradation, and tylosin was stable either in H_2O or 0.01M $CaCl_2$. Similar trends were also seen

for initial tylosin concentrations of 25 and 50 mg L^{-1} (Data not shown). Therefore, either 0.01M

CaCl$_2$ or H$_2$O can be used as a solvent for the analysis of tylosin.

 According to the study by Paesen (1995a), solution pH had significant influence on

tylosin stability. Tylosin loss was lowest at pH 7, with about 3% decrease within 100 h. At

higher pH (base conditions) or lower pH (acid conditions), greater tylosin loss occurred. For

example, at pH 4 less than 40% tylosin remained within 100 h, whereas tylosin was completely

degraded at pH 11 at the same time. However, Kolz et al. (2005) reported that tylosin was stable

for at least one month in Milli-Q water at pH 5.7 to 6.7, suggesting that tylosin was much more

stable. To determine how stable tylosin is at different pHs and how long stability remains,
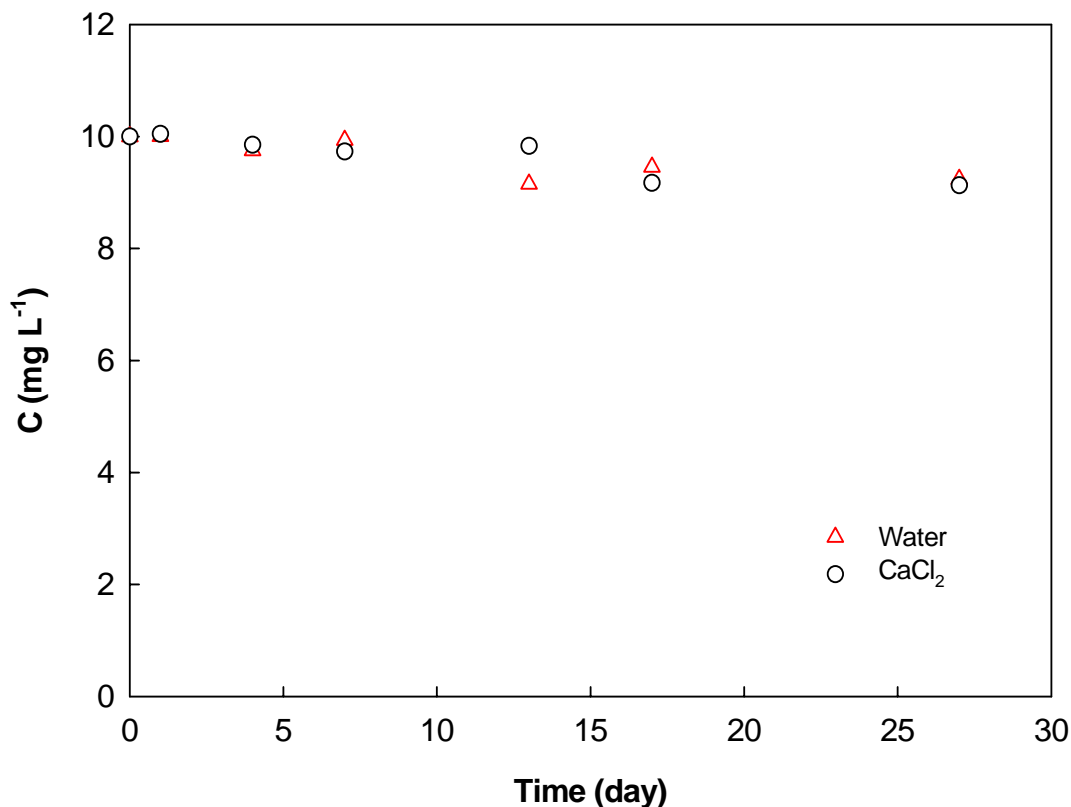
Fig. 2.4.   Tylosin stability in H$_2$O and 0.01 M CaCl$_2$ over time. Tylosin was dissolved in H$_2$O
and 0.01 M CaCl$_2$ at a concentration of 10 mg L^{-1} and kept at 25 ^{o}C for up to 27 days. Tylosin
concentration was quantified using HPLC.
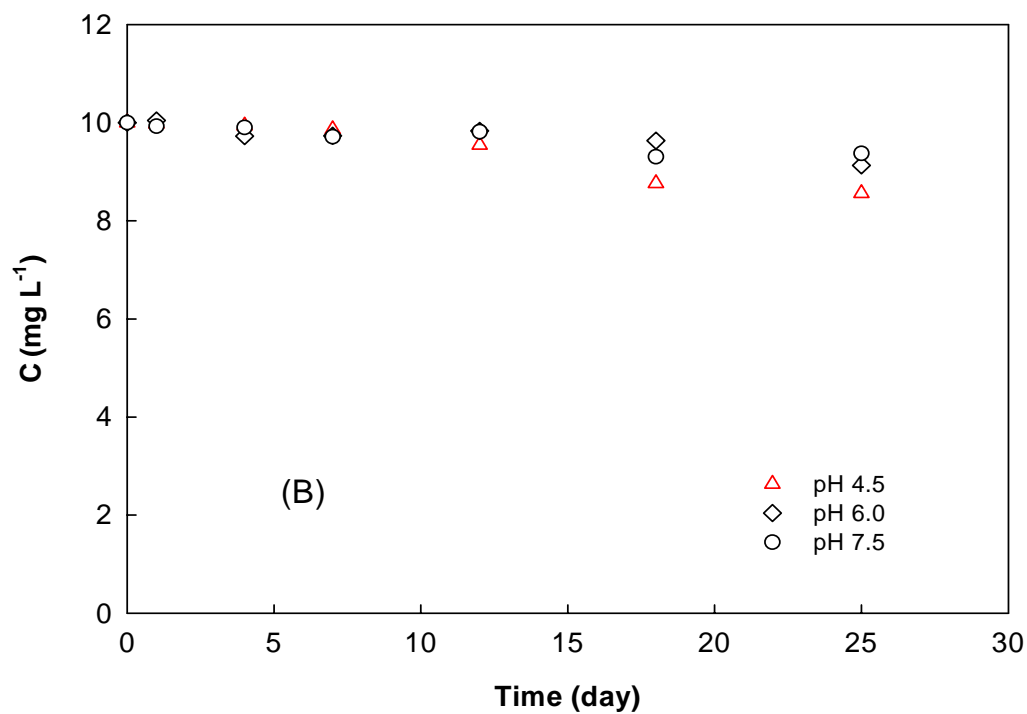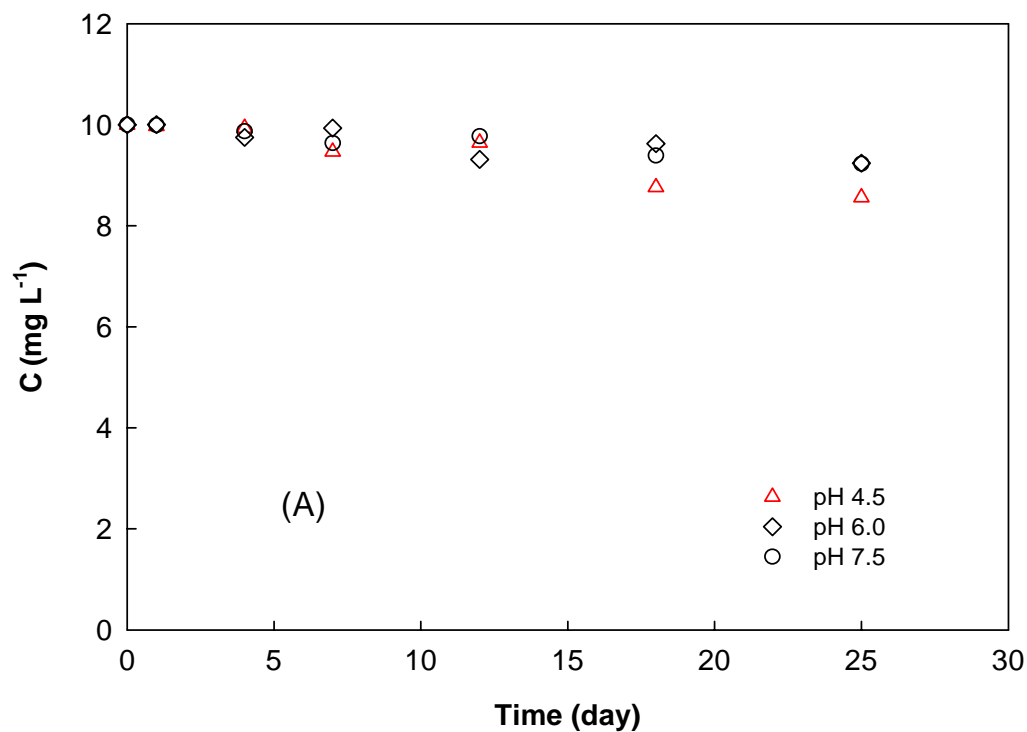
Fig. 2.5. Tylosin stability at pH 4.5, 6.0 and 7.5 in H_2O and 0.01 M $CaCl_2$. Tylosin at 10 mg L^{-1} in H_2O (A) or 0.01 M $CaCl_2$ (B) was kept at 25 ^{o}C and concentration measured at 1, 4, 7, 12,18 and 25 days using HPLC.

tylosin concnetrations at pH 4.5, 6.0 and 7.5 over 25 days were examined. As shown in Fig. 2.5, either in H_2O (A) or 0.01 M $CaCl_2$ (B) at a starting concentration of 10 mg L^{-1}, tylosin concentration was constant at pH 4.5, 6.0 or 7.5 within 12 days, showing no degradation. However, there was a slight decrease in tylosin concentrations afterwards, and a significant decrease was seen at pH 4.5 as compared to initial concentration as well as to concentrations at pH 6.0 or 7.5 at the same time points. Similarly, at higher initial concentrations of 25 and 50 mg L^{-1}, tylosin remained stable at pH 4.5, 6.0 or 7.5 within 12 days (data not shown).

Tylosin half life in 0.01 M $CaCl_2$ and H_2O at pH4.5, 6.0 and 7.5 was estimated according to the first-order kinetic model (Sparks, 2004) and the results are shown in Table 2.2 Tylosin half life in 0.01 M $CaCl_2$ (109 days) was similar to that in H_2O (115 days) at pH 4.5, but it appeared to be slightly longer in 0.01 M $CaCl_2$ (223 and 231 days) than in H_2O (213 and 216 days) at pH 6.0 and 7.5, indicating that tylosin was slightly more stable in 0.01 M $CaCl_2$ than in H_2O over longer time. However, tylosin half time was much lower at pH 4.5 as compared to that at pH 6.0 and 7.5, suggesting that tylosin degradation occurred at acid condition.

Table 2.2 Half life of tylosin in different solvents

Condition	water	0.01 M $CaCl_2$
pH	---------------$T_{1/2 \text{ (day)}}$-----------------	
4.5	115	109
6.0	213	223
7.5	216	231

Our study demonstrated that tylosin is stable at pH 4.5 to 7.5 within a short time of period (12 days), but degradation occurred over time. Tylosin stability shown in this study was longer than Paesen's study (3% loss in 100 hours) (Paceson et al., 1995a), but shorter than one month reported by Kolz (2005). The greater degradation found by Paesen et al. (1995a) may be attributed to potassium phosphate buffer or bacterial contamination in buffer. Tylosin

degradation under non-sterile condition was probably due to bacteria activity (Ingerslev et al., 2001). In our study, tylosin was placed in sterile H_2O or 0.01 M $CaCl_2$, thus excluding bacterial contamination. Regarding Kolz's study, because they did not provide any data, it is impossible to explain the reasons why tylosin was more stable than our study shown.   In terms of half life, tylosin half life in our study (Table 2.2) was slightly longer than the average of 200 days in unsterile pond water, sterile pond water and ultrapure water reported by Hu and Coats (2006) except for pH4.5.

2.3.3 Tylosin Stability at 4 ^{o}C and 25 ^{o}C

The temperature higher than 60 ^{o}C causes tylosin degradation both at pH 4.0 and 9.0 (Paesen et al., 1995), but there is no comparison of its stability between 25 ^{o}C (room temperature) and 4 ^{o}C. In this study, tylosin stability at 4 ^{o}C and 25 ^{o}C was compared. Results showed that at 4°C or 25 ^{o}C, there was no significant degradation either at pH 4.5, 6.0 or 7.5 (10 mg L^{-1} in 0.01 M $CaCl_2$) during the first 12 days (Fig. 2.6). After 12 days of incubation, tylosin at 25 ^{o}C was decreased slightly.

Thus, tylosin is as stable at 25 ^{o}C as at 4 ^{o}C so that short-term studies can be performed at 25 ^{o}C without concern of tylosin degradation since most tylosin sorption and desorption experiments are conducted in short times.

2.4 Conclusions

Several factors that affect tylosin stability were evaluated and following conclusions could be made: (1) Light has no effect on tylosin stability for 12 days, but degradation can occurs over time; (2) Tylosin in 0.01 M $CaCl_2$ had similar stability as in H_2O over a period of 27 days; (3) Tylosin was stable for 12 days between pH 4.5 and 7.5 but degradation occurred over longer time, especially at pH 4.5, and estimated half time of tylosin in $CaCl_2$ was slightly longer that in

H₂O; (4) Tylosin had similar stability at 25 ^{o}C as at 4 ^{o}C for 12 days but degradation occurred afterwards, especially at 25 ^{o}C.
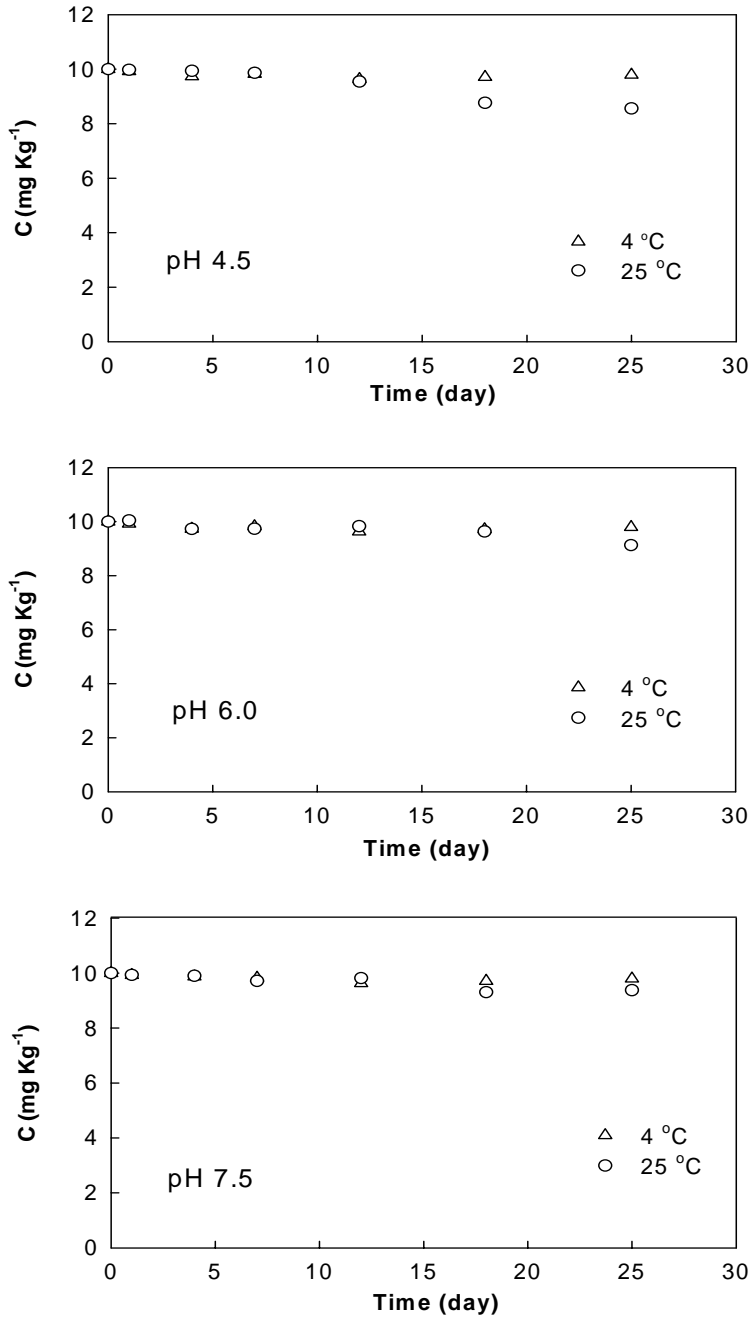
Fig. 2.6. Tylosin stability over time at 4 ^{o}C and 25 ^{o}C at pH 4.5, 6.0 and 7.5. Final concentration was measured using HPLC.

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CHAPTER 3

TYLOSIN SORPTION TO SOIL AS INFLUENCED BY PH AND ORGANIC MATTER AND ITS DESORPTION FROM SOILS

3.1 Introduction

Tylosin, a macrolid class compound, is one of the most widely used veterinary antibiotics in the United States, European Union (EU), Australian, New Zealand and several other countries. It has been administered to swine, beef cattle and chicken for disease prevention and control as well as growth promotion. The amount of tylosin administered in animals is high. For example, Denmark used 14,000 kg of tylosin in 1997 and UK 5144 kg of tylosin in 2000 (Loke et al., 2000; Smarmah et al., 2006). Due to the fact that tylosin likely enters into the environment through manure application first on agricultural lands, sorption and desorption behaviors of tylosin in soils are likely to play a major role in controlling the mobility and fate of these antibiotics.

Limited studies have reported that tylosin is strongly adsorbed in Danish soils (Rabolle et al., 2000). Adsorption partition coefficient (K_d) was found to range 8-11 for sandy soil and loamy sand soils, and 62-128 for two sandy loam soils. Additional study (Clay et al., 2005) on South Dakoda soils showed that Kranzburg soil (with 63.3 % silt, 29.1 % clay and 7.6 % sand) had higher adsorption than Badger soil (with 56.6 % silt, 31.0 % clay and 12.4 % sand). Similarly, tylosin adsorption on a Dutch clay loam soil was higher than that on a loamy sand soil (Laak, et al., 2006). These studies clearly suggest that soil with a higher percentage of silt and clay had higher sorption of tylosin than the soil with a higher percentage of sand.

Besides texture, soil pH and ionic strength were shown to affect tylosin sorption. Tylosin is a weak base, and pH may change the charge of both tylosin and soil particles, thus affecting

adsorption. Adsorption of tylosin was higher under weak acid conditions (pH 6). As pH

increased, sorption partition coefficient (K_d) decreased from 156 to 32 for the Dutch clay loam

soil and from 8.9 to 3.0 for the loamy sand soil (Laak, et al., 2006). Addition of an electrolyte

($CaCl_2$ or NaCl) more concentrated than 0.03 M to the solution led to decreased sorption of

tylosin by 3-fold (Laak, et al., 2006).

There has been no report in the literature so far about the effect of organic matter in soil

on tylosin sorption.   However, organic matter has been showed to impact the adsorption of other

antibiotics in soils. A recent study found that organic matter itself did not contribute to antibiotic

adsorption directly (Allison et al., 2005). For example, oxytetracycline was not sorbed on

cellulose and lignin, except slight adsorption on humic acid. Instead, organic matter likely

reduces adsorption of antibiotic to soil by hydrophobic partitioning (Kulshrestha et al., 2004) or

masking sorption sites (Pill and Laird, 2007), as seen in decrease in oxytetracycline adsorption to

Na- montmorillonite with increased concentration of humic acid (Kulshrestha et al., 2004),

suggesting that hydrophobic partitioning was involved. This was further confirmed by X-ray

diffraction analysis that humic acid was found to either masks sorption sites on clays or inhibits

interlayer diffusion of tetracycline to clays (Pill and Laird, 2007).

As compared to the sorption of tylosin in soils, there have been very few studies on

desorption of tylosin, and results were varied. A study by Clay et al. (2005) found that tylosin

desorption from South Dakota soil was low. In a 24-h period less than 0.2% of the total tylosin

sorbed to three soils was desorbed even at the high concentration. No tylosin was desorbed from

soils exposed to a low tylosin concentration. However, another study conducted by Rabolle et al.

(2000) indicated much higher desorption. Using similar desorption methods as Clay et al.(2005),

they found that 69% and 26%   of sorbed tylosin was released from Borris and Lunggaard loam

soils, respectively, whereas the desorption from South Dakota sandy loam soil was only 13-14%.

These studies indicated that the exact nature of adsorption and desorption of tylosin in soils are still not clear.

In this study, sorption characteristics of tylosin to three poultry litter-impacted Louisiana soils were studied. The effect of soil pH and organic matter on tylosin sorption was also determined. Moreover, tylosin desorption from the soils was evaluated in different pH condition, as well as extractable tylosin by organic solvent.

3.2 Materials and Methods

3.2.1 Soil Samples and Characterization

Three poultry litter-impacted pasture soils, Briley (loamy, siliceous, semiactive, Thermic Arenic Paleudults), Ruston (Fine-loamy, siliceous, semiactive, Thermic Typic Paleudults), and Savannah (Fine-loamy, siliceous, semiactive, Thermic Typic Fragiudults), were collected from northern Louisiana and used in this study. All three soils have been used for forage production of row crops and have been applied poultry litter. Soil samples were taken from 0-15 cm depth at each soil site. They were air-dried, grounded and passed through a 2 mm sieve before use. Soil pH was measured in deionized water with a soil/solution ratio 1:1 (m/m). Soil particle size analysis was conducted by using pipette method (Gee and Bauder, 1986). OM content was determined by the Wakley-Black method (Wakley, 1947). The cation exchange capacity (CEC) was measured by saturating the soil with 1 M ammonium acetate at pH 7, followed by distillation and titration (Soil Survey Laboratory Metheds Manual, 1996). Selected physical and chemical properties of these soils are given in Table 3.1.

3.2.2 Sorption Experiments

Tylosin sorption isotherms by three Louisiana soils were conducted at soil solution pH 4.5, 6.0 or 7.5. To do so, 2-g samples of Briley, Ruston, and Savannah soils were mixed with 19

Table 3.1 Selected physical and chemical properties of three sandy loams

Soil	pH	Sand	Silt	Clay	OM	CEC	Extractable P Bray II	Clay mineraology				
								Kao	Mic	Sme	Chl	IMS
		------------g Kg^{-1}-------------				cmolc Kg^{-1}	mg Kg^{-1}	--------- %---------				
Briley	5.8	660	208	132	69.9	9.7	2101	74	10	16	-	–
Ruston	5.9	716	130	154	69.8	10.8	2380	68	15	–	17	–
Savannah	6.8	524	343	133	69.3	9.1	4625	83	17	–	–	–

* OM, organic matter; CEC, cation exchange capacity; Kao, kaolinite; Mic, clay mica (illite); Sme, smectite; Chl, chlorite; IMS, randomly interstratified clay mica and smectite

ml 0.01 M CaCl$_2$ solution in a series of 25-ml centrifuge tubes and the mixtures were continuously shaken to make uniform soil suspensions. The pH of the suspension was repeatedly adjusted to 4.5, 6.0 and 7.5 (with 0.1M HCl or NaOH) until the overnight change in pH was < 0.2.

The pH adjusted suspensions were then spiked with a volume of tylosin stock solution to yield initial tylosin concentrations of 0, 5, 10, 20, 50, 100, or 200 mg L^{-1}, respectively. The spiked mixtures were shaken in a reciprocal shaker at 170 rpm for 24 h. After equilibrium, the mixture suspensions were centrifuged at 11000 rpm for 10 min and filtered through a 0.2 μm membrane filter, and filtrates were transferred to amber glass vials for HPLC analysis. Tylosin in filtrates was analyzed using an Agilent 1100 HPLC system, which was equipped with ODS column (250 x 460 mm i.d.) and 5 μm particles, and a mobile phase consisting of sodium perchlorate (2.25% m/v) at pH 2.5 and acetonitrile (60:40 v/v). The HPLC system was operated at a flow rate of 1.0ml min^{-1}, column temperature of 35 $^{\circ}$C, injection volume of 25 μl, and detection wavelength of 290 nm.

The amount of tylosin sorbed by soil was calculated by the difference between amount of tylosin spiked and tylosin in equilibrium solution, and was expressed as mg tylosin per kg of soil.

Freundlich Equation was used to model sorption data. Freundlich Equation is expressed as

$$q = K_f \bullet C^{1/n}$$

And its linear form is

$$\text{Log } q = \log K_f + 1/n \bullet \log C$$

Where q is the amount of tylosin sorbed, K$_f$ (the distribution coefficient), 1/n is the Freundlich linearization factor and C the equilibrium tylosin concentration.

In addition, in order to generate sorption at a wide range of soil pH, sorption experiment was also conducted with a single concentration of 100 mg L^{-1} tylosin in 0.01 M $CaCl_2$ solution with pH adjusted to 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0, respectively. The sorption partition coefficient (K_d) was calculated by amount of tylosin sorbed divided by equilibrium tylosin concentration after 24 h sorption.

Similar experiment was carried out with the three selected soils after organic matter being removed to determine the effect of organic matter on tylosin sorption. Organic matter in soils was removed by treating soils with 30% H_2O_2 in a boiling water bath with continuous agitation (Sheldrich et al., 1993). This treatment was repeated till no bubble formed. The soils were then washed with distilled water, dried at 60 ^{o}C, ground, and passed through the sieve of < 2 mm before use. All sorption experiments were conducted in duplicate.

3.2.3 Desorption and Extract Experiments

Tylosin desorption was carried out after sorption at initial concentration of 100 mg L^{-1} with the three soils for 24 h. The resultant tylosin-sorbed soils were collected for desorption experiments.

Tylosin-sorbed was desorbed by adding 20 mL 0.01 M $CaCl_2$ solution with pH adjusted to 4.5, 6.0 or 7.5, respectively. Desorption mixtures were continuously shaken in a reciprocal shaker at 170 rpm for 24 h. After equilibrium, the mixtures were centrifuged, filtered through a 0.2 μm membrane filter, and the filtrates were placed into amber glass vials for HPLC analysis. Desoprtion was repeated for 10 times, 24 hours each. The amount of tylosin desorbed was determined each time and corrected for the tylosin present in residue solution based on centrifuge tube, soil, and residual solution weights. Tylosin desorption was expressed as the percentage of amount of tylosin desorbed from soil relative to total tylosin sorbed by soil.

After desorption experiment using 0.01 M CaCl$_2$, the soils were also extracted for tylosin using organic solvent, methanol. The extraction was conducted by adding 20ml methanol in each of 25 ml centrifuge tubes, mixing on vortex mixer for 1 min and then vigorously shaking in a reciprocal shaker for 1 h, followed by centrifugation. Extraction was repeated 3-4 times until no tylosin was detected in supernatants. The supernatants were collected and passed through a 0.2 μm filter for quantification using HPLC as described before.

3.3 Results and Discussion

3.3.1 Tylosin Sorption Isotherms and pH Effect

  Tylosin sorption isotherms for Briley, Ruston, and Savannah soils are shown in Figs.3.1 to 3.3, respectively. The sorption curves were well characterized by the Freundlich equation ($R^2 = 0.98$ to 0.99) (Table 3.2). The distribution coefficient K_f was used to compare tylosin sorption. As shown in Table 3.2, Briley had the highest K_f for tylosin at K_f 170.0 L Kg^{-1} at pH 6.0, which indicates greater tylosin sorption, followed by Savannah with K_f of 142.8 L Kg^{-1} at pH 7.5, whereas Ruston soil had the lowest K_f of 92.6 L Kg^{-1} at pH 6.0, which indicates the lowest tylosin sorption among theses soils. Clearly, great differences existed among soils in sorption of tylosin.

    The results indicated sorption for tylosin by three Louisiana soils varied greatly (2 to 2.5 fold). This difference may be attributed to varying physical and chemical properties, particularly, increasing sorption with increasing clay and silt content (Allaire et al., 2006; Clay et al., 2005). With smaller size and larger area, clay and silt particles have higher sorption for antibiotics and other matter than sand, and may be the partial reason why Ruston soil had lower tylosin sorption than Briley and Savannah soils. Ruston soil had the highest sand content (71.6%) and lower silt content (13.0%), compared to Briley and Savannah soils with lower sand content of 66.0% and
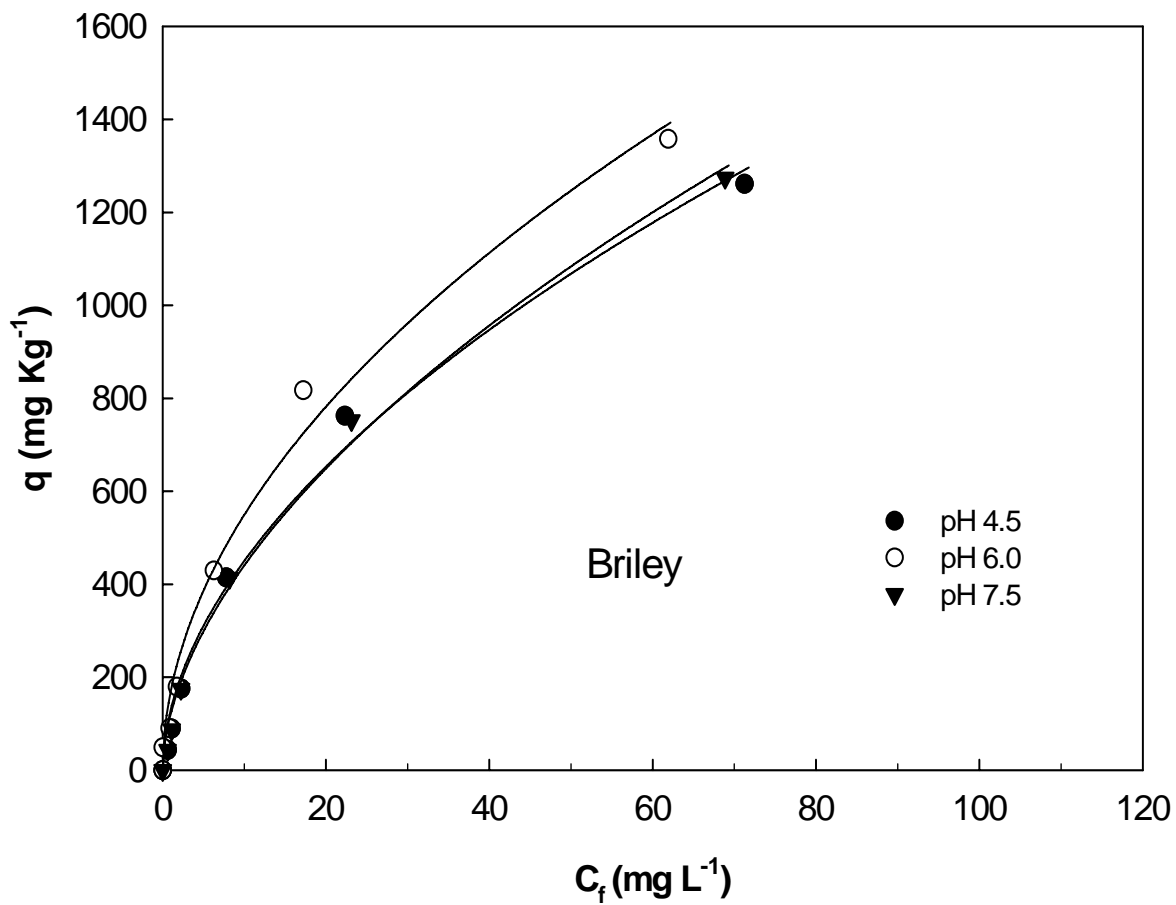
Fig.3.1 Tylosin sorption isotherms for Briley soil at different pHs. q = sorbed; C_f = final solution concentration (equilibrium concentration). Dots indicate experimental data and smooth lines indicate fittings using Freundlich model.
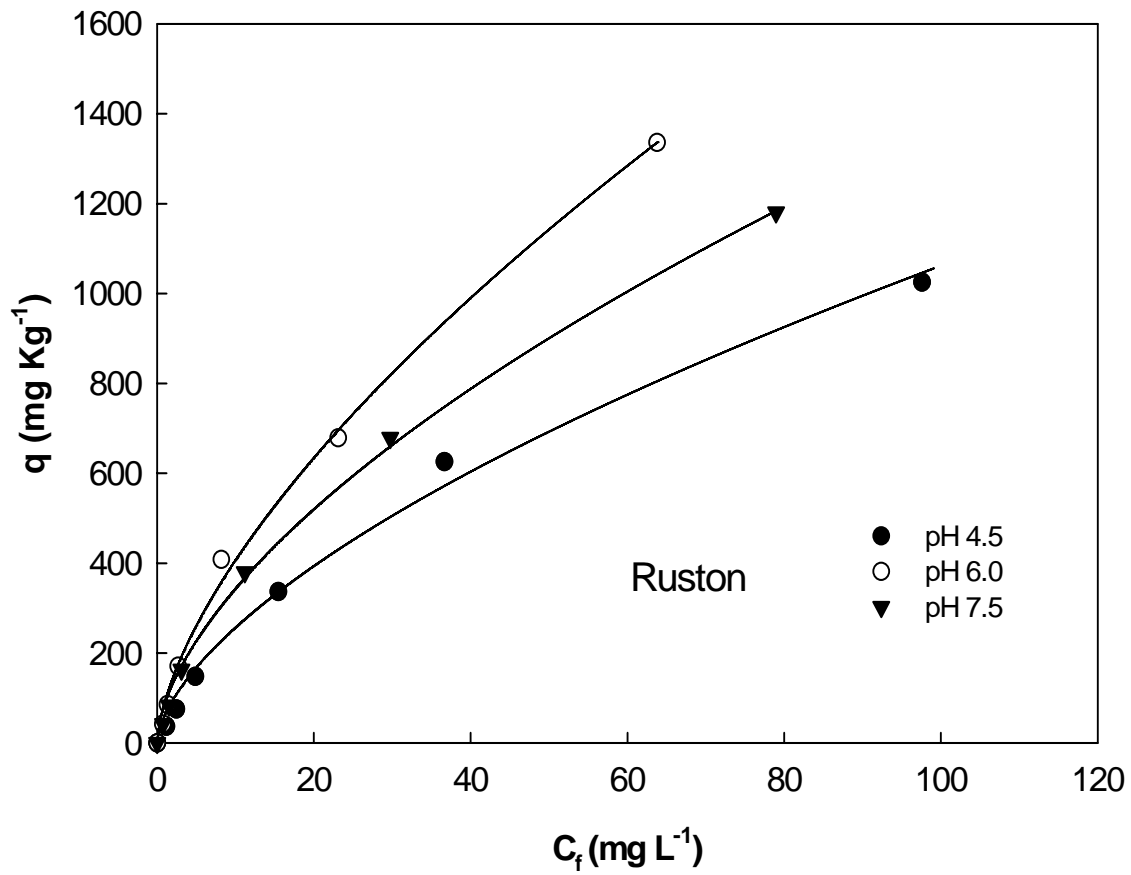
Fig.3.2 Tylosin sorption isotherms for Ruston soil at different pHs. q = sorbed; C_f = final solution concentration (equilibrium concentration). Dots indicate experimental data and smooth lines indicate fittings using Freundlich model.

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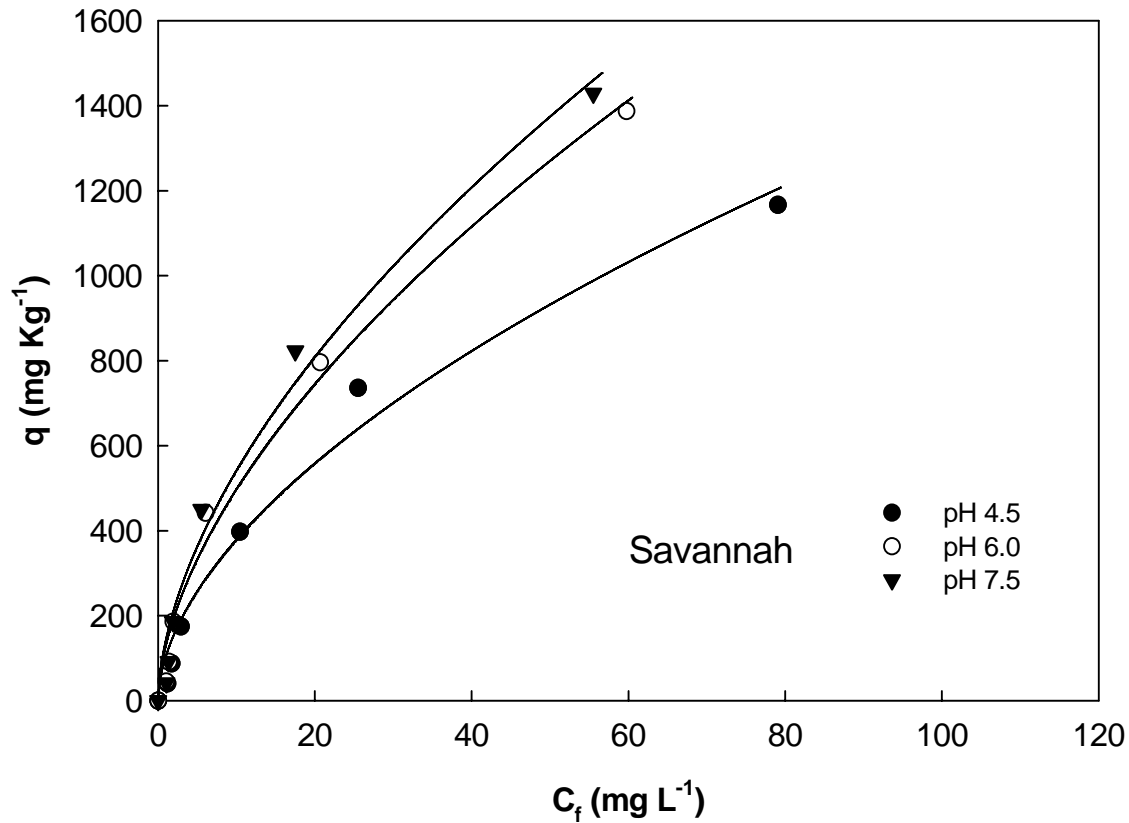
Fig.3.3 Tylosin sorption isotherms for Savannah soil at different pHs. q = sorbed; C_f = final solution concentration (equilibrium concentration). Dots indicate experimental data and smooth lines indicate fittings using Freundlich model.

Table 3.2 Tylosin sorption parameters by soils

Soil	pH	K_f (L Kg^{-1})	1/n	R^2
Briley	4.5	131.8 ± 12.0	0.53 ± 0.02	0.99
	6.0	170.0 ± 16.8	0.50 ± 0.03	0.99
	7.5	121.4 ± 9.2	0.55 ± 0.02	0.99
Ruston	4.5	62.0 ± 7.1	0.61 ± 0.03	0.99
	6.0	92.6 ± 10.2	0.64 ± 0.03	0.99
	7.5	86.5 ± 11.5	0.59 ± 0.03	0.99
Savannah	4.5	104.6 ± 13.5	0.59 ± 0.03	0.98
	6.0	130.1 ± 14.4	0.58 ± 0.03	0.99
	7.5	142.8 ± 18.8	0.57 ± 0.04	0.98

52.4%, but higher silt content of 20.8% and 34.3%, respectively (Table 3.1). Although Ruston

had slightly higher clay content (15%) than Briley (13%) and Savannah (13%), its much higher

sand content than Briley and Savannah, and much lower silt content than Briley and Savannah

were clearly unable to overwhelm its slightly advantage on clay content.   Similar results were

reported for other soils. For example, South Datoda Kranzburg, Waubay and Badger soils had

tylosin adsorption of K_f of 1510, 1290 and 1260 respectively, corresponding to their silt content

of 63.3%, 60.9% and 56.6% respectively (Clay et al., 2005). Higher adsorption on Minnesota

Webster soil was mainly due to its greater exchange capacity because of higher clay content

(34%) than Hubbard soil (10%) (Chander et al, 2005). A study by Thiele-Bruhn et al. (2004)

suggested that sulfonamide adsorption was also correlated with soil particle size, in agreement

with our studies. Beside the particle size, the clay mineraology was likely another important

factor in determining tylosin sorption. This could be especially the case for higher sorption by

Briley than Savannah. Briley has much higher sand content (66%) than Savannah (52.4%), and

much lower silt content (20.8%) than Savannah (34.3%), but it had higher tylosin sorption.

However, Briley contained 16% of smectite in its clay whereas Savannah had no smectite.

Smectite clay minerals included montmorillonite, which was shown to have 2 to 3 times higher

tylosin adsorption than illite, and 10 times higher than kaolinite (Bewick, 1979).

Tylosin sorption isotherms also indicated that soil pH affects tylosin sorption (Figs. 3.1 to

3.3, and Table 3.2). At pH 6.0, both Briley and Ruston soils had the highest amount of sorption,

with K_f values of 170.0 and 92.6 L Kg^{-1}, respectively. For Savannah soil, the highest sorption

was at pH 7.5 with K_f of 142.8 L Kg^{-1}, which was close to K_f 130.1 L Kg^{-1} at pH 6.0. Lowest

sorption occurred at pH 4.5 for Ruston and Savannah soil, with K_f values of 62.0 and 104.6 L

Kg^{-1} respectively, whereas Briley had the lowest sorption at pH 7.5 although sorption did not

showed much difference between pH 4.5 and 7.5. The lowest sorption represented a 33.0%, 23.1% and 26.8% reduction in sorption K_f for Briley, Ruston and Savannah soil, respectively, as compared to their highest K_f at pH 6.0 or 7.5.

Although sorption isotherms were conducted at three pHs, the pH ranges were too large to accurately reflect sorption as a function of pH. To better determine how pH affects tylosin sorption, additional sorption experiments were conducted with a single initial tylosin concentration between pH 4 and 9 with smaller pH increments. The results were shown in Fig.3.4 to 3.6. In this case, K_d, sorption partition coefficient, was calculated after 24 h sorption. For Briley soil, it exhibited greater sorption at pH 4.0, then dropped at 4.5 but peaked at pH 6.0 with K_d about 46 L Kg^{-1} (Fig.3.4). At pH higher than 7.0, tylosin sorption dramatically decreased, with K_d reduced to less than 10 at pH 9.0. Unlike Briley soil, tylosin sorption for Ruston soil did not show higher sorption at low pH and was maximal at pH 6.0 to 7.0, with K_d about 30 L Kg^{-1} (Fig.3.5). Outside this pH range, tylosin sorption decreased significantly.   At pH 8.5 K_d was less than 10 L Kg^{-1}. Savannah soil showed a similar tylosin sorption pattern to Ruston soil with peak sorption at about pH 6.0 to 7.5 and K_d about 65 L Kg^{-1} (Fig.3.6). Outside this pH range, tylosin sorption decreased considerably. At either pH 4.5 or 8.5, tylosin sorption dropped to K_d 25 L Kg^{-1}, the lowest level.

Our study demonstrated that pH is a major factor affecting tylosin sorption by these poultry litter-impacted soils. Tylosin sorption was reduced by 60 to 75% at the lowest pH as compared to its maximum sorption except for Briley. The higher sorption was at pH 4 and around 6.0 for Briley soil, 6.0 to 7.0 for Ruston soil, and 6.0 to 7.5 for Savannah soil. Except for Briley, higher or lower pHs than 6.0-7.0 range resulted in dramatic reduction in tylosin sorption.
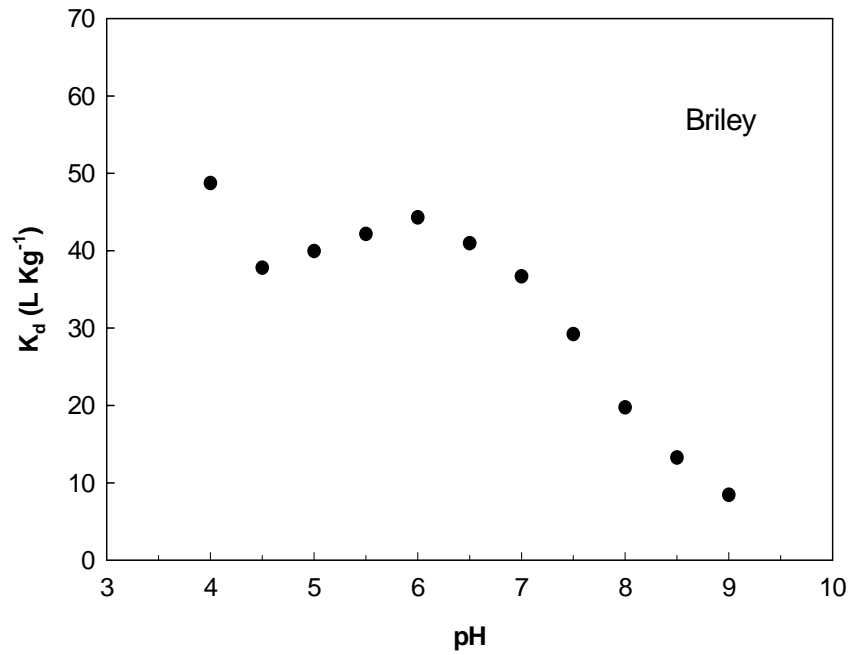
Fig.3.4 Tylosin sorption partition coefficient K_d as a function of pH for Briley soil.
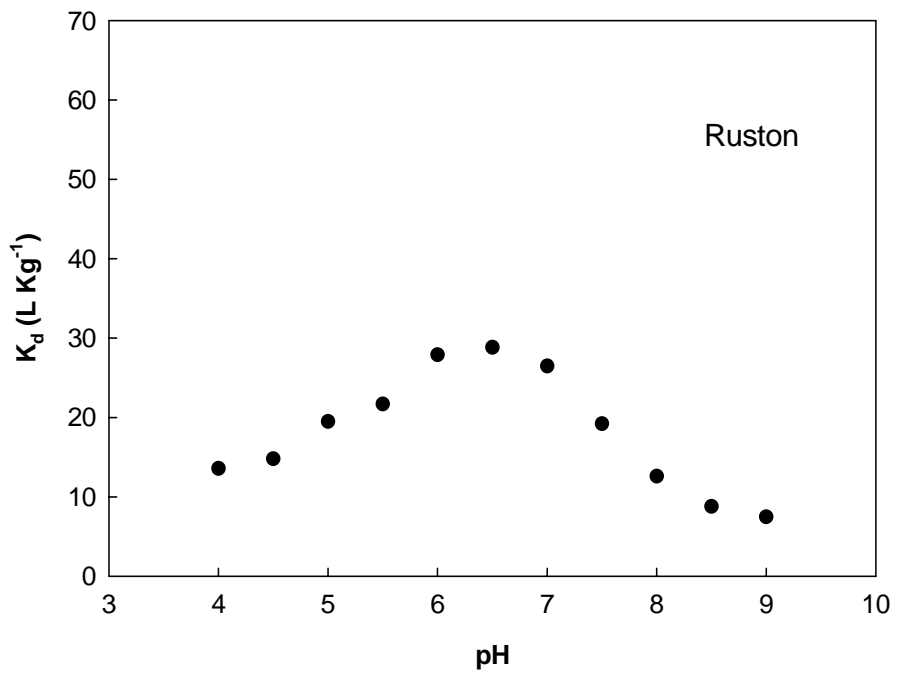
Fig.3.5 Tylosin sorption partition coefficient K_d as a function of pH for Ruston soil.
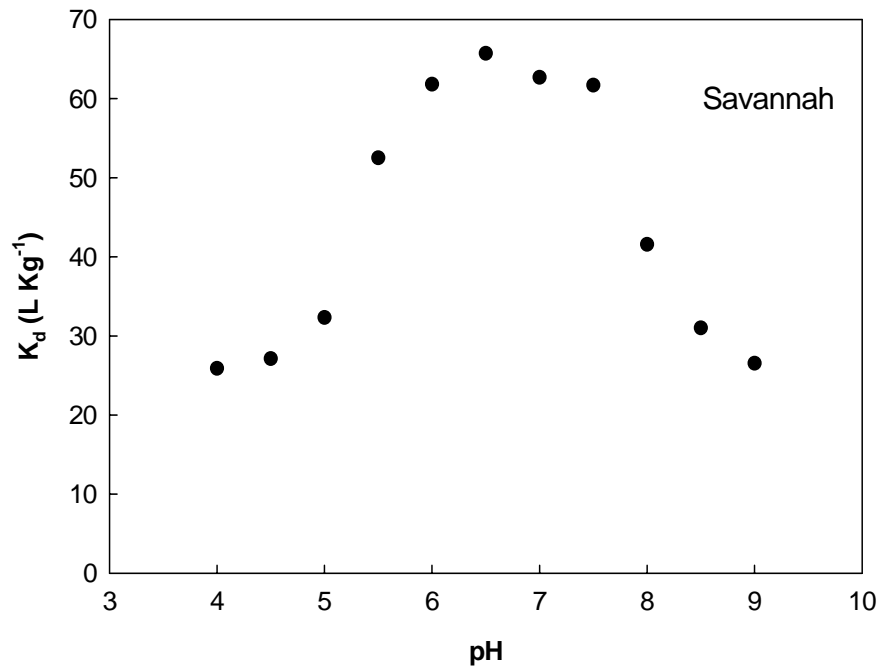
Fig.3.6 Tylosin sorption partition coefficient K_d as a function of pH for Savannah soil.

Effect of pH on tylosin sorption likely reflects changes in charge of soil particles as well as tylosin at different pHs. It has been suggested that electrostatic forces were the mostly responsible for antibiotic sorption to charged soil surfaces (Holten et al., 2000), and pH affects charges of both soil particles and antibiotics. At pH > 6.0 – 6.5, the R-COOH of soil organic matter (Pk_a ~5.8-6.0) increases its dissociation and becomes negatively charged surface (Sparks, 2003) but tylosin (Pk_a 7.73) loses its positively charged site from protonation of dimethyl-amino group on sugar moiety (Block et al., 2003). Therefore, as pH increases to > 6.0 to 7.5, tylosin sorption decreases. At pH < 5.8 – 6.0, organic matter or mineral surface becomes more positively charged, so does tylosin, the repulsive force of positively charged surface had sorption decreased for Savannah and Ruston. As for Briley, due to its higher content of smectite [the point of zero charge (Pzc) of 2.5 for montmorillonite], mineral surface is negatively charged at pH > 2.5. This negatively charged surface could still adsorb positively charged tylosin, which

may partially explain why the highest tylosin sorption can be achieved at low pH 4.0 to soil Briley. A closer look at chromatograph also revealed the presence of a small peak at pH 4.0. According to Nalda et al., (2006), this small peak most likely represents tylosin B transformed from tylosin A at this pH. This suggests that the high sorption of tylosin at pH 4 could be due to the transformation of tylosin A to tylosin B. Such phenomenon was not observed with other pHs and soils.

This study demonstrated that pH is an important factor in determining sorption for tylosin to soil, but related studies are very limited. One study reported that tylosin sorption to Dutch clay loam and sandy loam soil was the highest at about pH 6.0, and decreased with pH increase (Laak et al., 2006). Our study showed a similar trend in that sorption was highest about pH 6.0. However, the study by Laak et al. (2006) did not show sorption at pH lower than 6.0. In contrast, broader pH range was evaluated in our study.

Our study also suggests that pH effect on tylosin sorption curve is different among soils. Tylosin sorption to Briley soil decreased with pH increase, while to Ruston and Savannah soils sorption increased with pH, reaching a maximum around pH 6.5, and decreased with further pH increase. The sorption pattern for Briley soil has been observed in the sorption of other antibiotics. For example, sorption of sulfachloropyridazine to Dutch clay loam soil and sandy loam soil decreased from a maximum at low pH to a lowest level at high pH (Boxall et al., 2002, Laak et al., 2006). Similar trends have been seen in sorption of oxytetracyline to Connecticut iron oxide-rich soils (Figueroa et al., 2005) and sorption of tetracycline to Florida soils (Sassman et al., 2005). Besides soils, sorption of sulfamethazine and tetracycline to clay minerals montmorillonite and kaolinite also exhibited similar trends (Gao et al., 2005; Figueroa et al., 2004). As for tylosin sorption curve by pH in Ruston and Savannah soils, we have not see any

similar reports either in other antibiotics or soils and clays, but this sorption curve was observed in sorption of oxytetracycline to iron oxides (Figueroa et al., 2004). We suspect that the pH-dependent change due to high organic matter content in these soils may contribute these observed sorption phenomena, and further studies are needed to address this difference.

3.3.2 Effect of Organic Matter on Tylosin Sorption in Soils

Our study showed that there was an inflection point of maximum tylosin sorption at pH 6.0 for Briley soil, and maximal sorption at pH 6.0 to 7.0 for Ruston and Savannah soils (Figs. 3.4 to 3.6). Various studies have shown that soil organic matter can have significant effect on sorption behavior of organic compounds (Thiele, 2000; Allison et al., 2005; Gu et al., 2008). In order to evaluate impact of organic matter on tylosin sorption, the soils were treated with H_2O_2 for removing organic matter and tylosin sorption was carried out under similar pH range.

Tylosin sorption to soils after organic matter being removed had two significant changes (Figs.3.7 to 3.9). First, removal of organic matter resulted in dramatic increase of tylosin sorption. After organic matter was removed from soils, maximum amount of tylosin sorption to Briley soil was increased by about 8-fold, to Ruston soil by 4-fold, and to Savannah soil by 2-fold as compared to the soils without organic matter being removed (Figs.3.4 to 3.6). Second, greater amount of tylosin sorption was achieved at acid pH 4 for Briley and Ruston, and tylosin sorption decreased dramatically with pH increase, dropping to the lowest level at about pH 9. For Savannah soil, tylosin sorption under acid conditions was increased slightly, reaching maximum at about 6.6. Above pH 7, tylosin sorption to the three soils after organic matter being removed decreased dramatically, as compared to those soils with organic matter.

Our study suggests that organic matter in soil contributes to reduce sorption of tylosin. This is different from the early report that higher organic matter was the main cause that the

fertile soils (2.4% organic matter) had higher sorption of sulfapyridine than infertile soils (1.6% organic matter) (Thiele, 2000). Other studies revealed complicity of relationship between organic matter and antibiotic sorption (Allison et al., 2005; Gu et al., 2008). In organic matter, cellulose and lignin had no affinity for oxytetracycline, but humic acid had low affinity for oxytetracycline (Allison et al., 2005). Humic acid has been found to interact with hydrous Al oxide (HAO), reducing its sorption of tetracycline (Gu et al., 2008). Under different concentrations of humic acid, sorption of oxytetracycline to Na- montmorillonite exhibited opposite patterns (Kulshrestha et al., 2004). At a lower concentration of humic acid (1 mg L^{-1}), oxytetracycline sorption increased, while at a higher concentration of humic acid (10 mg L^{-1}) oxytetracycline sorption decreased, showing hydrophobic partitioning in the solution of clay-associated humic acid. Moreover, X-ray diffraction analysis by Pils et al. (2007) found that humic acid reduced interlayer sorption of tetracycline to smectites by either masking sorption sites on surface of clay minerals or inhibiting interlayer diffusion of tetracycline. In this study, the relatively high organic matter content (~ 70 g Kg^{-1}) of these soils could have attributed to reduction in sorption of tylosin to the three Louisiana soils through similar mechanisms. It is reasonably assumed that once organic matter was removed, sorption of tylosin onto soils was just like onto clay minerals. This appeared to be the case for Briley and Ruston soils, which showed generally decrease trend of tylosin sorption as pH increased from 4 to 9, a trend similar to those reported for Dutch soils (Laak et al., 2006) In addition, although the three soils used in this study have approximately the same amount of organic matter, their response in sorption to the removal of organic matter was different, with Briley soil showing an 8-fold increase, Ruston a 4-fold increase and Savannah a 2-fold increase. This could partly reflect their difference in clay minerals as seen in Table 3.1. Furthermore, even with same organic matter content, its
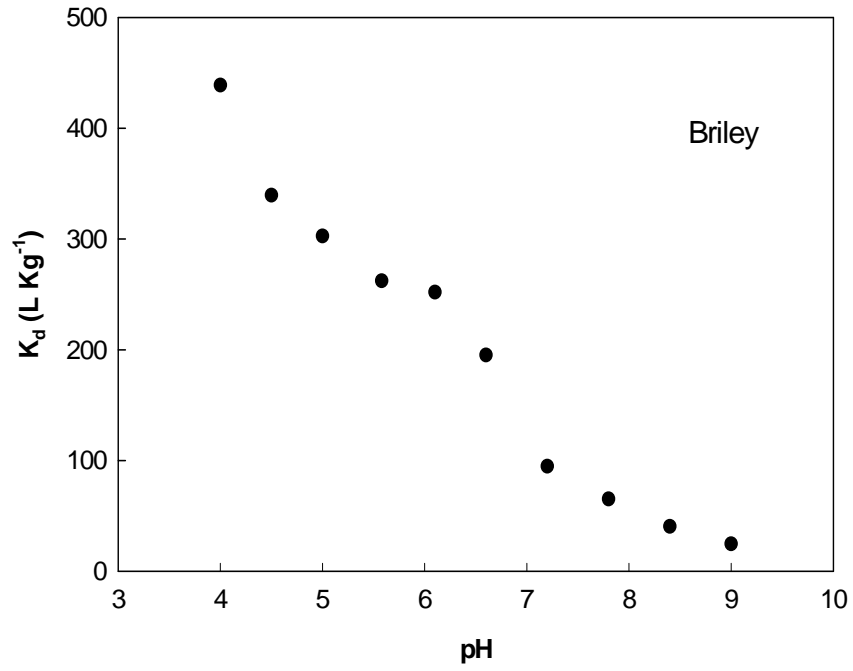
Fig.3.7. Tylosin sorption partition coefficient K_d for Briley soil as a function of pH after organic matter being removed.
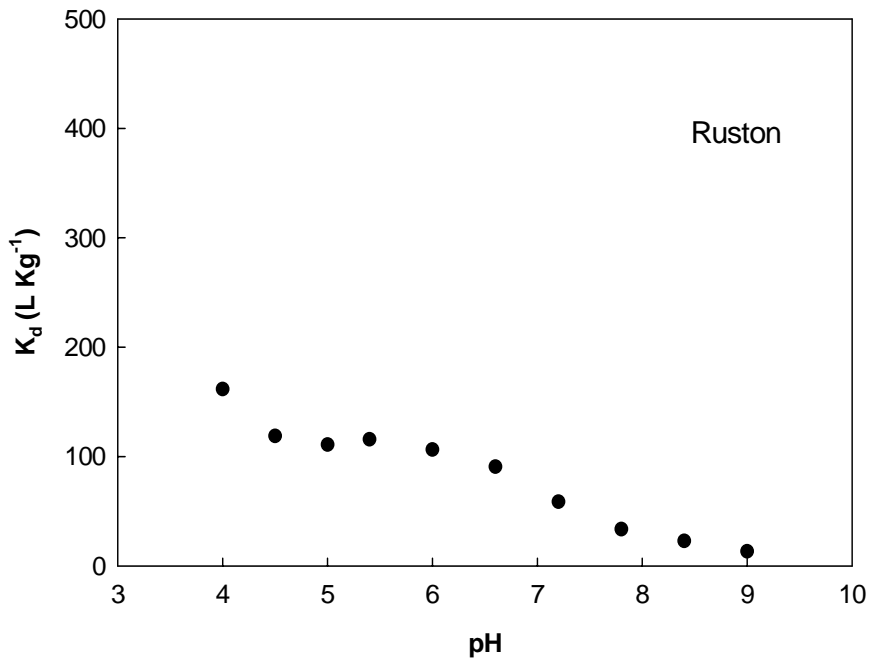
Fig.3.8. Tylosin sorption partition coefficient K_d for Ruston soil as a function of pH after organic matter being removed.
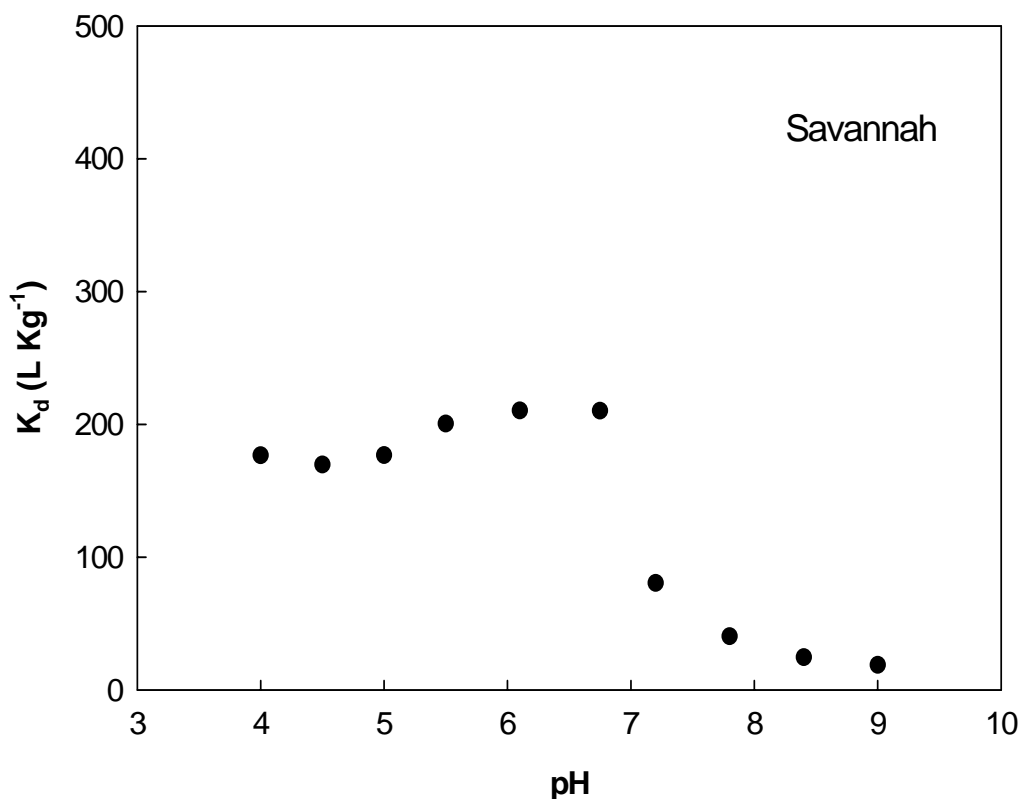
Fig.3.9. Tylosin sorption partition coefficient K_d for Savannah soil as a function of pH after organic matter being removed.

composition (cellulose, lignin, humic acid) may be different, which would likely further account

for differences in tylosin sorption as a function of pH.

3.3.3 Tylosin Desorption from Soils

Three soils exhibited different desorption patterns by 0.01 M $CaCl_2$ (Figs. 3.10 to 3.12).

Approximately about 43.5 to 94.5 % tylosin was desorbed after 10 times of desorption (once a

day), with the highest desorption from Briley soil, followed by Ruston soil, and the least

desorption from Savannah soil. Desorption occurred very quickly, with the most tylosin being

desorbed within about five time, but minor tylosin desorption continued for 10 times. This study

demonstrated that less tylosin was desorbed from Savannah soil than from Ruston soil and Briley

soil, suggesting that tylosin in Savannah soil has lower mobility compared to Ruston and Briley soils.

It can be seen from Figs. 3.10, 3.11 and 3.12 that tylosin desorption was affected by pH, and effect of pH on tylosin desorption varied among soils. For Briley, pH only had little effect on tylosin desorption. Desorption at pH 4.5 and 6.0, which was 74.3% and 73.5% respectively after 10 desorptions in the 0.01 M $CaCl_2$ solution, was slightly higher than desorption at pH 7.5, which was 67.6% in the same condition. Tylosin desorption appeared to flat out starting after 8 desorptions at pH 7.5, but desorption at pH 4.5 and 6.0 still continued even with the 10th desorption. By contrast, pH had greater effect on tylosin desorption from Ruston soil. Desorption at pH 4.5 was the highest, with 95% of tylosin desorbed at the 10th in the 0.01 M $CaCl_2$ solution, followed by desorption at pH 6.0 with 79.6% of tylosin desorbed in the same condition. The least desorption occurred at pH 7.5 with 60.7% of tylosin desorbed. Like in Briley, tylosin desorption from Ruston also flatted out at pH 7.5 starting at the 3rd in the 0.01 M $CaCl_2$ solution, but desorption at pH 4.5 and 7.5 still continued in the 10th equilibrium solution. As for tylosin desorption from Savannah, pH had slightly different impact as compared to Briley and Ruston. Desorption was highest at pH 4.5 with 55.4% of tylosin desorbed at the 10th desorption in the 0.01 M $CaCl_2$ solution, followed by desorption at pH 7.5 with 49.2% of tylosin desorbed in the same condition. The least desorption occurred at pH 6.0 with 43.5% of tylosin desorbed. No additional tylosin desorption was observed at the 4th in the 0.01 M $CaCl_2$ solution at pH 6.0 and 7.5, but the 6th desorption at pH 4.5.

Very few studies have been conducted on tylosin desorption from soil. One study found that only 0.2% of the tylosin sorbed on three South Dakota soils was desorbed by using 0.01 M $CaCl_2$ for 24 h (Clay et al., 2005). Another study showed higher desorption from Borris soil
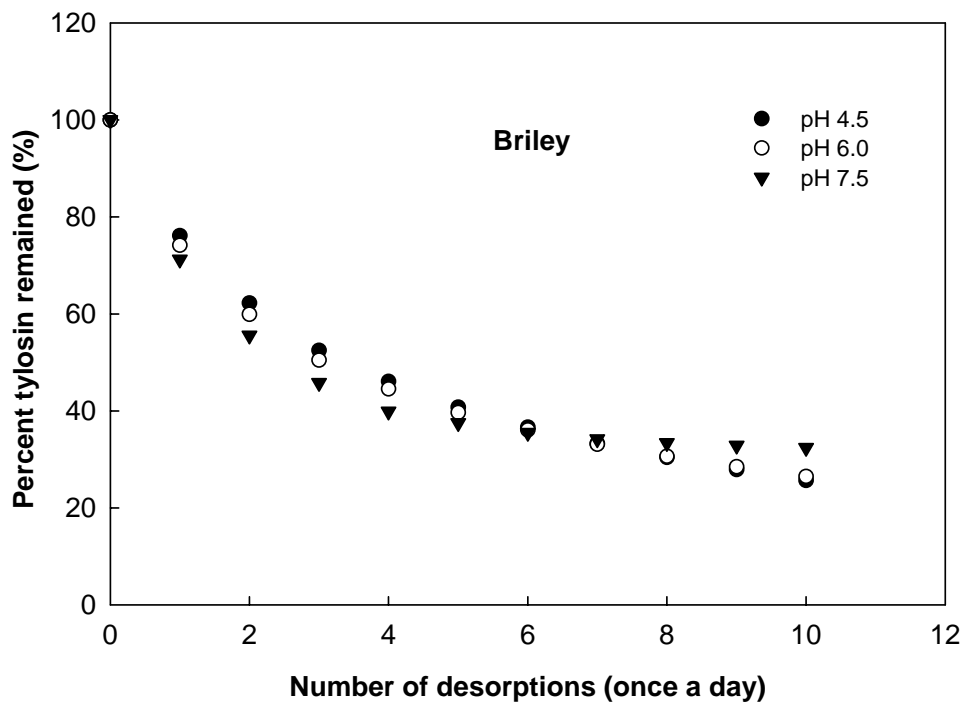
Fig.3.10. Tylosin desorption by 0.01M CaCl$_2$ for Briley soil
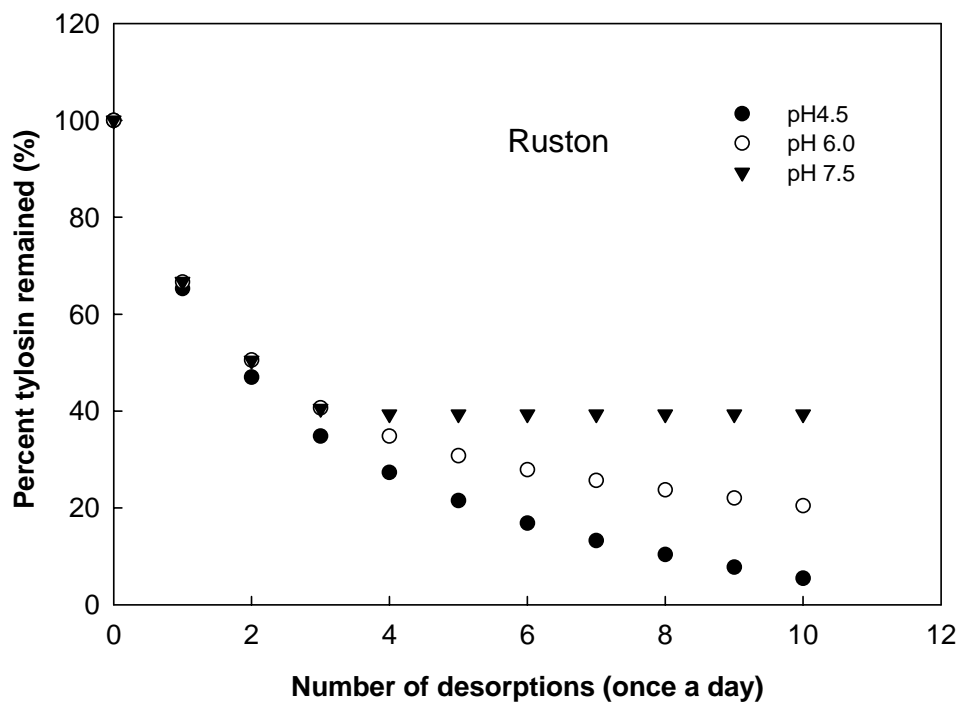
Fig.3.11. Tylosin desorption by 0.01M CaCl$_2$ for Ruston soil
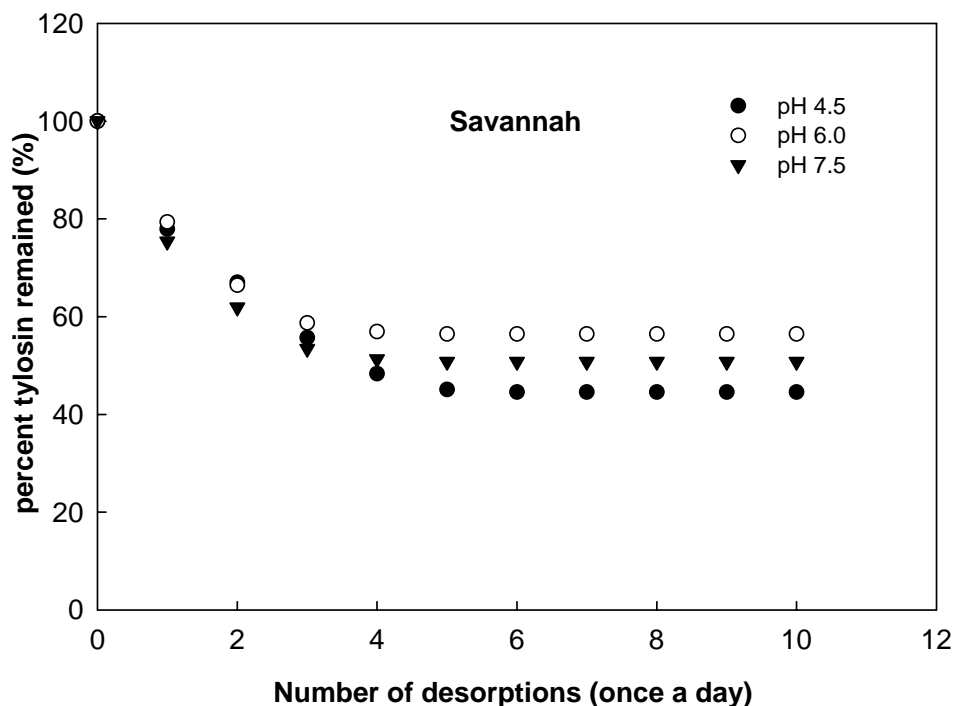
Fig.3.12. Tylosin desorption by 0.01M CaCl$_2$ for Savannah soil

69% and from Lunggards soil 26%, from low sandy loam soil 13-14 % (Rabolle et al., 2000).

Clearly, tylosin desorption varied from soil to soil. However, desorption period was only 24 h in

these two studies, and tylosin desorption may occur beyond this time. Our study is in agreement

with these two studies in that soil is a major factor in determining tylosin desorption. Moreover,

our study extended desorption period till 10 days when most desorable tylosin has been desorbed,

giving a more detailed view of tylosin desorption.

Our studies for the first time indicated that tylosin desorption was affected by pH, and

this has an application in prevention of tylosin release. Higher percentage of tylosin desorption

from soil indicats its higher mobility in soil. Raising soil pHs likely decreases its mobility in soil.

For example, tylosin adsorption on Ruston soil was as high as 79.6.1% at pH 6.0, but it was

reduced to 60.7% at pH 7.5, a change of pH from 6.0 to 7.5 resulting in reduction of tylosin

release by about 20%. This could be a way to prevent tylosin release from soil into water bodies

by reducing tylosin desorption from soil. However, this approach is limited for some soils like Briley from which tylosin desorption was relatively stable from pH 4.5 to 7.5.

3.3.4 Tylosin Recovery by Organic Solvent

The desorption studies using 0.01M $CaCl_2$ showed some tylosin was not desorbed from the soils. To determine the total recovery of tylosin, extraction of tylosin was also performed using methanol, an organic solvent, after tylosin desorption by 0.01 M $CaCl_2$. As indicated in Table 3.3, unaccounted tylosin from 0.01 M $CaCl_2$ desorption can be extracted from soils by methanol, but recovery rate differed among soils and pHs. Depending on pH and amount of tylosin remained in soil after desorption by 0.01 M $CaCl_2$, tylosin extraction by methanol contributed 1 to 20% of total recovery for Ruston soil, 15 to 29% for Briley soil, and 25 to 30% for Savannah soil. Ruston soil had the lowest total unrecovered rate after 0.01 M $CaCl_2$

Table 3.3 Tylosin recovery from methanol extraction

Soil	pH	q (mg Kg^{-1})	Desorbed by 0.01 M CaCl2 (%)	Extracted by methanol (%)	Total Unrecovered (%)
Briley	4.5	1261 ± 10	82	15	3
	6.0	1358 ± 6	79	18	3
	7.5	1273 ± 9	67	29	4
Ruston	4.5	1025 ± 25	98	1	1
	6.0	1336 ± 12	82	18	0
	7.5	1180 ± 3	61	20	19
Savannah	4.5	1166 ± 2	56	25	19
	6.0	1387 ± 13	43	23	34
	7.5	1428 ± 21	49	30	21

* q is the tylosin sorbed after 24 h equilibrium in 0.01 M $CaCl_2$ solvent.

desorption and methanol extraction, ranging from 0 to 1% at pH 4.5 and 6.0, indicating that the unaccounted tylosin after 0.01 M $CaCl_2$ desorption was tightly sorbed on soil, instead of being degraded. However, there was 19 % of tylosin not recovered by methanol at pH 7.5. Similarly,

Briley soil also had lower total unrecovered rate after 0.01 M $CaCl_2$ desorption and methanol extraction, ranging from 3 to 4% between pH 4.5 and 7.5. Savannah soil had the highest total unrecovered rate after 0.01 M $CaCl_2$ desorption and methanol extraction, ranging from 19 to 34%, indicating that tylosin was much more tightly sorbed on Savannah soil and not recoverable by methanol extraction, as compared to Briley and Ruston soils.

Although we could not rule out the possibility of some unrecovered tylosin in sorption by Savannah soil being degraded, it is unlikely that this could happen considering similar experimental condition was maintained with Savannah as with Ruston and Briley. On the other hand, lower recovery rates have been reported with methanol and other organic solvents (Kolz et al., 2005, Rabolle et al, 2000). The studies with other antibiotics also showed that tightly bound antibiotic compounds may be extracted by other method, such as pressurized liquid extraction (Stoob, et al., 2006; Schlusener et al., 2003). Nevertheless it appears that unextractility of antibiotic compounds in Danish soils is common (Rabolle et al, 2000). The total tylosin recovery, including desorption by 0.01 M $CaCl_2$ and extraction by methanol, was between 66% (Savannah soil at pH 6.0) to 100% (Ruston soil at pH 6.0). This was better than from the recovery rate of 61 to 81% obtained from soil column study (Rabolle et al., 2000). As seen in this study, tylosin recovery rate was mainly determined by soil in the order of Ruston > Briley > Savannah, and to a less extent by pH as higher pH (> 7.0) appeared to reduce recovery rate, likely due to the strong sorption between positively charged tylosin and negatively charged soil mineral and organic matter surfaces.

3.4. Conclusions

In this study, tylosin sorption to three Louisiana soils at different pHs was characterized. The role of soil organic matter in sorption was also evaluated. Tylosin sorption was well

described by the Freundlich equation, with sorption following the order of Briley > Savannah > Ruston. Tylosin sorption to soils was affected by pH, with greater sorption at acid pH 4.0 for Briley, and at pH 6 to 7 for Ruston and Savannah. Soil organic matter decreased sorption of tylosin, especially sorption under acid conditions. Tylosin desorption from soils differs greatly among soils, with Ruston soil the highest, followed by Briley soil, and Savannah soil the lowest. Similar to sorption, tylosin desorption is greatly affected by pH, with higher desorption under acid conditions (pH 4.5), and lower desorption under weakly acid (pH 6.0) or basic (pH 7.5) conditions.

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CHAPTER 4

TYLOSIN SORPTION AND DESORPTION CHARACTERISTICS OF SELECTED CLAY MINERALS

4.1 Introduction

Tylosin, a macrolide antibiotic, has good antibacterial activity against a broad-spectrum of pathogenic organisms such as gram-positive bacteria, some gram-negative bacteria, vibrio, spirochete, coccidian, etc., and it is widely used for disease control, disease prevention and growth promotion in animal production. For example, Denmark used 14,000 kg tylosin in 1997 and UK 5144 kg of tylosin in 2000 (Loke et al., 2000; Smarmah et al., 2006). Tylosin is produced by fermentation of Streptomyces strain. Structurally, it consists of a substituted 16-membered lactone ring, an amino sugar (mycaminose), and two neutral sugars (mycinose and mycarose). Tylosin is a mixture of tylosin A, tylosin B, tylosin C and tylosin D (See Fig 1.1). Tylosin is a weak base with pKa 7.73, and remains relatively stable at pH 7 (Paesen et al., 1995a), but is prone to photodegradation (Halling-Sorensen et al., 2003).

Soil is a heterogeneous mixture of airs, water, inorganic and organic solids, and microorganisms. In a typical silt loam soil ideal for plant growth the solid component in the surface horizon represents about 50% of the volume (45% mineral and 5% organic matter), air comprises about 20-30%, and water makes up the remaining 20-30% (Sparks, 2003). Inorganic solids contain numerous clay minerals, which are naturally occurring materials composed primarily of fine-grained minerals. Clay minerals are the main components of soil that determin its physical and chemical properties, such as soil shrinkage, CEC value, and sorption for water, nutrients and antibiotics. Soil sorption was found to be determined by clay minerals. For

example, Dutch clay loam soil with higher content of clay and silt had greater tylosin adsorption than loam sandy soil with higher content of sand (Laak, et al., 2006). There are approximately 30 different types of clay minerals, and can be classified into various groups based on layer formation, isomorphic substitution and layer charge characteristics (Sparks, 2003). Among those, kaolinite, montmorillonite, and clay mica (illite) along with vermiculite and chlorite are common clay minerals found in most soils.

Clay minerals have strong sorption for large varieties of organic and inorganic contaminants, thus receiving more and more attention in the removal of environmental contaminants. Clay minerals are widely used to adsorb heavy metals from waste water. Montmorillonite, bentonite, and illite, were applied to remove such heavy metals in aqueous solution as Fe^{2+} (Schultz and Grundl, 2004), Co^{2+} (Shahwan et al., 2006), Ni^{2+} (Wang et al., 2007), Cu^{2+} and Zn^{2+} (Veil and Alyuz, 2007) and B^+ (Karahan et al., 2006). Montmorillonite, illite and kaolinite have been also used to adsorb pesticides such as dichloro-diphenyl-trichloroethane (DDT), dieldrin and heptachlor from aqeous pesticidal solutions (Huang and Liao, 1970). Recently, montmorillonite clays have been used to remove antibiotics, antioxidants, mold inhibitors and other organic compounds from poultry litter aqeous leachats. The addition of montmorillonite reduced the leachte toxicity (EC_{50}) (50% effective concnetration) by 127% at day 7 as compared to at day 1 (Gupta, et al., 2005).

Modified clay minerals have even greater capacity and wider spectrum for sorption of other complicated chmiecals. Recent organo-clays, synthesized by the ion exchange of sodium in Wyoming Na-montmorillonite with surfactants Octadecyltrimethylammonium bromide (ODTMA), dodecyldimethylammonium bromide (DDDMA), had hydrocarbon sorption for

diesel (1.2-7.2 g/g), castrol hydraulic oil (1.3-3.6 g/g) and valvoline super diesel engine oil (1.3-3.6 g/g) (Carmody, et al., 2007). Montmortillonite modified with different organic cations showed a remarked increase in sorption of herbicide simazine, with K_f (sorption coefficient) from 28-47 for the unmodified to 96-138, 400-753, and 10,000 for the modifed clay minerals (Cruz-Guzman et al., 2004). Similarly, modofied Na-rich Wyoming montmorillonite and Ca-rich Arizona montmorillonite removed 95% of herbicides terbuthylazine, diuron and MCPA [(4-chloro-2-methylphenoxy) acetic acid], initially present in aqeous solutions, in constrast to less than 15% by the unmodofied montmorillonite (Ceils et al., 2007). In addition, modifed clay minerals have been used for the removal of antibiotics from water. A micelle-clay systems of montmorillonite complexed with benzyldimethylhexadecylammonium (BDMHDA) removed 96-99.9% of tetracycline antibiotics (chlortetracycline, oxytetracline and tetracycline) and sulfonamide antibiotics (sulfamethoxazole, sulfisoxazole, and sulfamethizole) from water solutions (Polubesova et al., 2006). Another compound, didodecyldimethylammonium bromide (DDAB) charged vesicle-montmorillonite complex could also efficiently remove 92-100% of sulfentrazone, imazaquin and alachlor and 60% of atrazine from the contaminated water (Undabeytia et al., 2008).

Tylosin sorption to clay minerals was rarely studied. The only one study by Bewick (1979) performed tylosin sorption to clay minerals, as well as desorption in a phosphate buffer of potassium salts, which is known to effect expandability of clay minerals (Scott et al., 1987). In addition, sorption capacity was evaluated in fairly short time period of 4 h, and sorption kinetics of tylosin was not quantified. With widespread use of tylosin in animal industry and poor knowledge of its sorption and desorption on clay minerals as well as increasing potentials of

direct use of clay minerals for environmental clean-up, it is the purpose of this study to characterize the tylosin sorption kinetics of selected clay minerals as well as tylosin desorption.

4.2 Materials and Methods

4.2.1 Clay Minerals and Chemicals

Three clay minerals, montmorillonite, kaolinite and illite, were purchased from the Clay Minerals Society Source Clay Repository (West Lafayette, IN, USA) and used in this study. The selected properties of clay minerals were given in Table 4.1. Reagent grade tylosin as tylosin tartrate was purchased from Sigma-Aldrich, Inc (St. Louis, MO, USA). Selected physical and chemical properties of tylosin are given in Table 4.2. Methanol (HPLC grade), sodium perchloride (HPLC grade), and acetonitrile (HPLC grade) were obtained from Fisher scientific Inc (Pittsburgh, PA, USA).

Table 4.1 The Selected properties of clay minerals

Clay minerals	Specific surface ($m^2 g^{-1}$)	CEC (meq Kg^{-1})
Montmorillonnite	83.8[a]	844 [a]
Illite	11.0[b]	170[b]
Kaolinite	10.1 [a]	20 [a]

a (Van Olphen and Fripiat, 1979); b (O'Loughlin., 2000)

4.2.2 Sorption and Desorption Experiments

The sorption and desorption of tylosin with clay minerals were conducted in 0.01 M $CaCl_2$ matrix using a batch method. Based on preliminary studies on sorption capacity, the initial concentrations of tylosin of 300, 600 and 4000 mg L^{-1} were used for sorption with

kaolinite, illite and montmorillonite respectively. Clay minerals (0.5g) were weighed into a

series of 25-ml preweighed centrifuge tubes, followed by adding 20 mL of 0.01 M $CaCl_2$

Table 4.2 The selected properties of tylosin

Molecular formula	$C_{46}H_{77}NO_{17}$
Appearance	A white or slightly yellow crystalline power
molecular weight	916.1
Solubility	5 mg ml^{-1} (water 25 ^{o}C)
Stability	Solution are stable at pH 4-9
pKa	7.73
UV absorbance	282nm

From Paesen et al. (1995b and c) and McFarland et al. (1997)

solution containing initial tylosin concentration as described above. The tubes were capped,

mixed using vortex mixer for 2 min, and then continuously shaken in a reciprocal shaker at 170

rpm, 25 ^{o}C (25 $\pm$ 0.5 ^{o}C) for 10 min, 30 min, 1 h, 3 h, 10 h, 24 h, and 48 h.   At each time

interval, the mixtures were centrifuged at 11000 rpm for 10 min.   The supernatants were filtered

through a 0.2 μm membrane filter, and the filtrates were placed in amber glass vials for HPLC

analysis.

The tylosin desorption experiments were carried out following adsorption by clay

minerals. After 24 h of sorption, the supernatant was decanted. The tubes with clay mineral

residues were weighed to determine the amount of the residual supernatant. A 20 ml 0.01 M

$CaCl_2$ tylosin-free solution was added in the centrifuge tubes, and the clay mineral residues with

tylosin adsorbed were resuspended using a vortex mixer for 2 min first and then shaken in a

reciprocal shaker at 170 rpm for 24 h. All experiments were conducted in duplicate.

Tylosin in adsorbing and desorbing solution was analyzed using an Agilent 1100 HPLC

system (Agilent Technologies, Palo Alt, CA, USA) equipped with ODS column (250 x 460 mm

i.d.) and 5 μm particles. The mobile phase consisted of sodium perchlorate (2.25% m/v) adjusted to pH 2.5 with hydrochloric acid, and acetonitrile (60:40 v/v). The flow rate was set at 1.0 ml/min, and the column was operated at 35 ^{o}C. The injection volume was 25 μl. The detection was carried out at a wavelength of 290 nm. A series of standards were prepared and analyzed with samples in the same HPLC run.

Adsorption (q_t) was calculated by difference between initial concentration and final concentration at a specific time interval and expressed as unit tylosin adsorbed per unit of clay minerals. The sorption data were further fit with power function equation,

$$q_t = at^b$$

Its linear transformation is

$$\ln q_t = \ln a + b \ln t$$

Where q_t = the amount (mg Kg^{-1}) of tylosin adsorbed on clay at time t;

t = reaction time (h);

a = power function model rate constant;

b = power function model constant; (Dalal, 1974);

Amount of tylosin desorption was calculated from the tylosin released into the desorbing 0.01 M CaCl$_2$ solution from clay minerals with tylosin adsorbed.

4.3   Results and Discussion

4.3.1 Tylosin Sorption Kinetics

Sorption kinetics of tylosin by the three clay minerals were shown in Figs. 4.1 to 4.3. In general, montmorillonite adsorbed tylosin more quickly than illite and kaolinite. For example, sorption to montmorillonite reached 91% of the maximal amount in 0.5 h, whereas sorption to illite and kaolinite just reached 79% and 34% of maximal amount respectively in the same

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period. Kaolinite adsorbed tylosin more slowly than montmorillonite and illite. To compare tylosin sorption rate, power function model, which has been used to describe kinetics of various processes in soil (Havlin and Westhall, 1985; Ye et al., 2006), was used to fit sorption kinetic data. As shown in Table 4.3, kinetics of tylosin sorption to three clay minerals were well described by the power function, with R^2 ranging from 0.94 to 0.99. Sorption rate (a) was in the order of montmorillonite > illite > kaolinite, indicating that montmorillonite had the fastest tylosin sorption rate, whereas kaolinite had the slowest sorption rate. On the other hand, the constant b, which had the order of kaolinite > illite > montmorillonite, was negatively correlated with adsorption, as suggested by Havlin and Westhall. (1985). Besides rate of sorption, montmorillonite had the highest amount of tylosin sorption, with sorption reaching about 126.3 g Kg^{-1} clay mineral, followed by illite, with its sorption of 5.2 g Kg^{-1} clay mineral after 48 h. Kaolinite had the lowest amount of sorption at about 1.1 g Kg^{-1} clay mineral after 48 h. Clearly, the three clay minerals had different tylosin sorption rates and amounts. Based on 48 h sorption experiments, sorption partition coefficient (K_d) was calculated and results were also shown in Table 4.3. The K_d values were 150.5 for montmorillonite, 10.9 for illite and 3.9 for kaolinite. This translated a montmorillonite's 13 times higher adsorption than illite, and a 38 times higher sorption than kaolinite.

This study demonstrated high amount of tylosin sorption to clay minerals with the order of montmorillonite > illite > kaolinite and maximum tylosin sorption could be reached at 48 h. Our study showed different results from what was reported by Bewick (1979). Bewick (1979) found longer than 4 h reaction did not affect amount of tylosin sorption by these three minerals, while the much of sorption was within 10 h in our study. This was clearly not the case for kaolinite, which had greater sorption of tylosin after even 10 h. In addition, sorption of tylosin by montmorillonite in 0.01 M $CaCl_2$ as in our study was much higher (126.3 g Kg^{-1}) than that
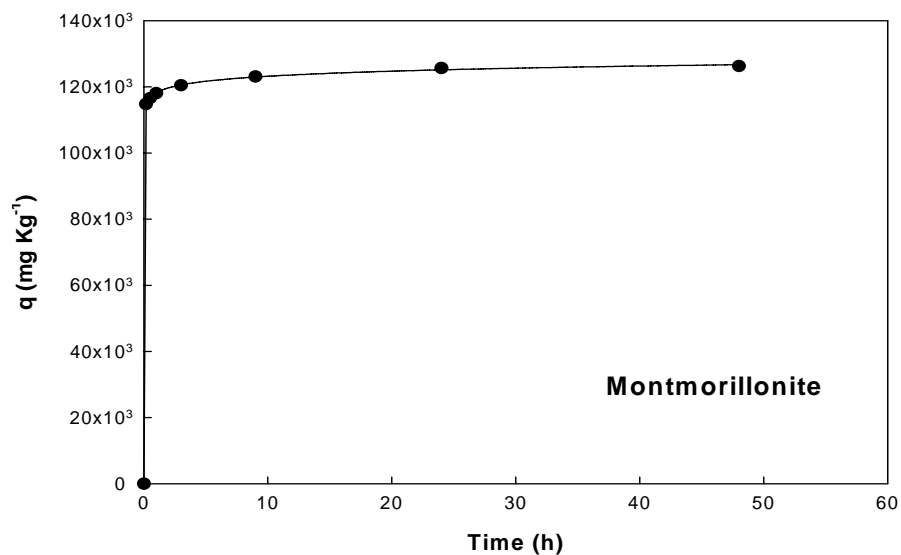
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Fig.4.1 Tylosin sorption kinetics for montmorillonite. Solid circles represent experimental observations, and line represents the model fitting by power function $q_t = at^b$.
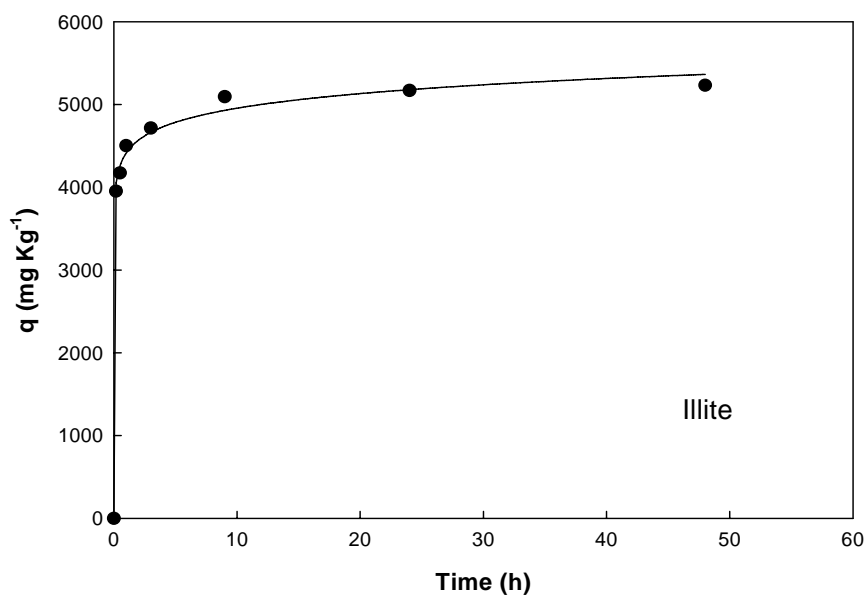
Fig. 4.2. Tylosin sorption kinetics for illite. Solid circles represent experimental observation, and line represents the model fitting by power function $q_t = at^b$.
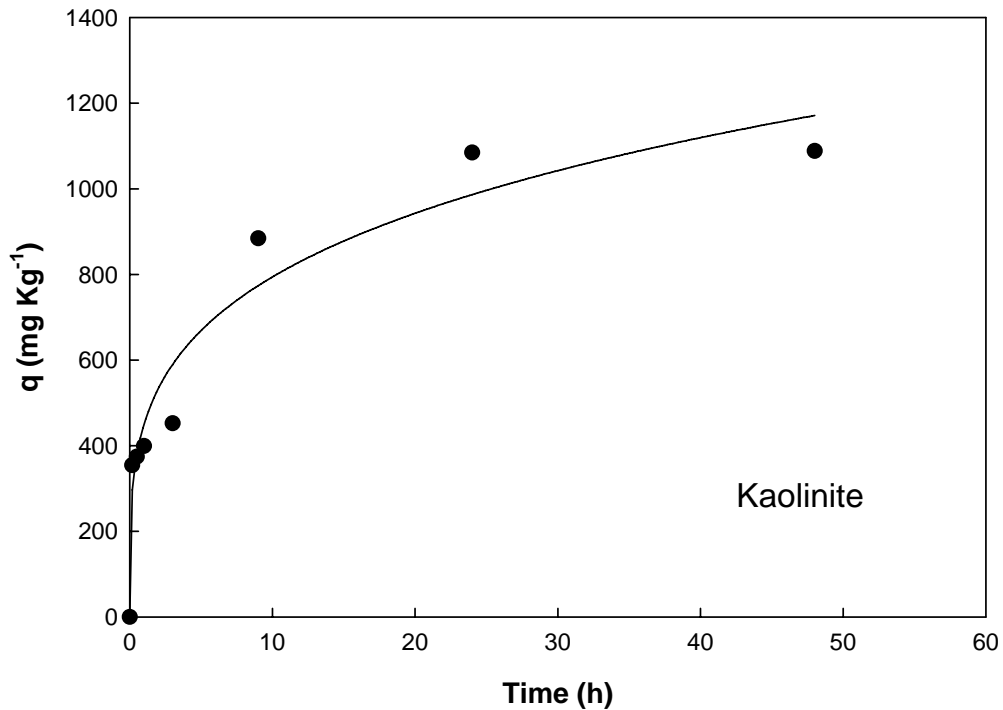
Fig. 4.3. Tylosin sorption kinetics for kaolinite. Solid circles represent experimental observation, and line represents the model fitting by power function $q_t = at^b$.

Table 4.3 Parameters of tylosin sorption to clay minerals

Clay mineral	Derived by power function equation $q_t = at^b$			K_d (L Kg^{-1})*	Q (g kg^{-1})*
	a	B	R^2		
Montmorillonite	118218 ± 146	0.018 ± 0.0005	0.99	150.5	126.3
Illite	4411 ± 48	0.051 ± 0.0046	0.99	10.9	5.2
Kaolinite	448 ± 45	0.248 ± 0.0338	0.95	3.9	1.1

*Based on sorption time was 48 h.

(65 g Kg^{-1}) reported in pH 8.0 phosphate buffer by Bewick (1979), suggesting that tylosin

sorption in 0.01 M CaCl$_2$ was likely less limited as compared to that in phosphate buffer. The

amount of tylosin sorption by illite and kaolinite were slightly lower. These differences could

also be attributed to the clay sources used in two studies.

It is not well documented why clay minerals have different tylosin sorption, but studies

from sorption of other antibiotics demonstrated that surface area and cation exchange capacity

(CEC) in clay minerals were the major factors to determine antibiotic sorption (Figueroa et al.,

2004; Gao et al., 2005). Clay minerals such as montmorillonite with higher sorption capacity

had more surface area as well as higher cation exchange capacity (CEC) than clays as kaolinite

with lower sorption (Table 4.1). Higher surface and cation exchange observed in

montmorillonite than illite and kaolinite are due to its expandable structure consisting of

aluminum octahedral sheet sandwiched by silica tetrahedral sheet. Illite has similar structure as

montmorillonite but its interlayer charge is balanced by K$^+$, making it nonexpendable. By

contrast, nonexpendable kaolinite consists of one aluminum octahedral sheet and one silica

tetrahedral sheet. Higher surface area and cation exchange capacity were highly correlated to

higher adsorption of antibiotics such as sulfonamide, tetracycline, and herbicides such as

pentachloronitrobenzene, chlorothalonil and isoprothiolane by montmorillonite than kaolinite

(Gao et al., 2005; Figueroa et al., 2004; Fushiwaki et al., 2001). Montmorillonite with higher

adsorption of tetracycline than kaolinite was found to have a 25 times higher surface area than

kaolinite's (Figueroa et al., 2004). Montmorillonite with higher adsorption of sulfonamide had

50 times higher cation exchange capacity than kaolinite's (Gao et al., 2005). Clearly, higher

sorption of tylosin by montmorillonite than illite and kaolinite, was also likely attributed to its

higher surface area and cation exchange capacity.

4.3.2 Tylosin Desorption

As shown in the tylosin sorption study that clay minerals strongly sorbed tylosin, it is of great interest to determine the stability of tylosin sorbed on clays. To that end, the tylosin-sorbed clays were washed with 0.01 M $CaCl_2$ for 24 h, and the desorption percentage was determined. As shown in Table 4.4, less than 10% of tylosin sorbed in montmorillonite was desorbed, whereas about 42.6% of sorbed tylosin was desorbed from illite. Tylosin sorbed on kaolinite had medium desorption (about 19.3%). This suggests that tylosin adsorbed by montmorillonite was more strongly held, but less strongly by illite, possibly due to interlayer space of the former.

Table 4. 4 Tylosin desorption from clay minerals in 0.01 M $CaCl_2$

Clay mineral	Sorption (mg Kg^{-1})	Desorption (mg Kg^{-1})	Desorption (%)
Montmorillonite	125671 ± 395	13021 ± 106	9.7
Illite	5169 ± 30	2254 ± 5	42.6
Kaolinite	1084 ± 10	218 ± 2	19.3

The results were consistent with those observed by Bewick (1979), who conducted tylosin desorption using phosphate buffer to wash tylosin-sorbed clays for 4 h, and found that tylosin desorption from montmorillonite was the lowest (7.4%) among the three clay minerals, followed by desorption from kaolinite (25%), and highest desorption from illite (30%). Overall, these results were similar indicating desorption power of 0.01 M $CaCl_2$ was similar to that of K alts of phosphete buffer even though Ca^{2+}, K^+ phosphate and Chloride could have different degree of interactions with interlayer and edge surfaces of these clay minerals.

4.4 Conclusions

In this study tylosin adsorption to clays montmorillonite, illite and kaolinite, and desorption was studied. The results demonstrate that rate and amount of tylosin sorption to clay

minerals are in the order of montmorillonite > illite > kaolinite, and adsorption is well descibed

by power functioin equation. For desorption, a proportion of sorbed tylosin can be desorbed

using 0.01 M $CaCl_2$ from clay minerals and desorption is in the order of illite > kaolinite >

montmorillonite, suggesting that some tylosin sorbed in clay minerals is not tightly bound

especially illite and kaolinite surfaces, which are primiarily planar as compared to the interlayers

of montmorillonite.

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CHAPTER 5

SUMMARY AND CONCLUSIONS

Tylosin is one of the widely used antibiotics in animal production for disease treatments, prevention, and control as well as growth promotion. There has been an increasing concern that tylosin could be released into soil through manure application and then find their way into river and underground water source, thus causing environmental contamination. The contamination to the environment by tylosin is mainly through manure application in farming land, and is largely controlled by sorption of tylosin on soil and desorption from it. However, tylosin sorption and desorption by soil were poorly understood, making it impossible to evaluate its risks to the environment and solve environmental problems. Moreover, tylosin sorption and desorption by clay minerals were rarely studied, restricting their use in environmental clean-up of tylosin. Therefore, the purpose of this study was to determine (1) the stability conditions for studying sorption and desorption of tylosin, (2) sorption and desorption by three Louisiana soils as well as effect of pH and organic matter on tylosin sorption, and (3) tylosin sorption to mineral clays and desorption.

To determine stability conditions for tylosin study, tylosin was evaluated under different conditions: light (exposure to light and dark), solvents (H_2O and 0.01M $CaCl_2$), pH (4.5, 6.0 and 7.5), and temperature (4 ^{o}C and 25 ^{o}C) for fixed periods, and amount of tylosin was quantified by high performance liquid chromatography (HPLC). The results showed that tylosin was stable in sealed vials for 12 days under light and dark condition, but slight degradation occurred afterwards, and significant degradation occurred in open vials. As for solvents, tylosin in 0.01M $CaCl_2$ was as stable as in H_2O during 27 days of experimental period, showing no difference between these two solvents. As for pH, tylosin was stable for 12 days at pH 4.5, 6.0 and 7.5, but

degradation occurred afterwards with higher degradation at pH 4.5 than at pH 6.0 and 7.5. For temperature, tylosin was stable at 25 $^\circ$C for 12 days as at 4 $^\circ$C, but longer time storage resulted in a slight degradation as compared to storage at 4 $^\circ$C.

To determine tylosin sorption and desoprtion, tylosin was adsorbed to three poultry litter impacted Louisiana Soils (Briley, Ruston and Savannah) in 0.01 M $CaCl_2$ solutions and desoprtion was carried out by washing soils with same solution. In addition, adsorption was carried out in soils with organic matter removed. The amount of tylosin was quantified by HPLC. The results showed that tylosin adsorption characteristics were well described by the Freundlick equation ($q = K_f \cdot C^{1/n}$), with $R^2 = 0.98$ to 0.99. Briley had the highest tylosin adsorption, followed by Savannah, and Ruston lowest. Tylosin adsorption to soils was affected by soil pH, with maximum adsorption at acid pH (4.0) for Briley, and at weak acid to neutral pH (6 to 7) for Ruston and Savannah. Removal of organic matter dramatically increased tylosin adsorption, especially under acid pH condition. Tylosin desorption differed greatly among soils, with Ruston highest, followed by Briley, and Savannah lowest. Tylosin desorption was also greatly affected by pH, with higher desorption under acid conditions (pH 4.5), lower desorption under weak acid (pH 6.0) or basic (pH 7.5) conditions. Tylosin undesorbed from soils by 0.01M $CaCl_2$ can be recovered by methanol treatments, showing its stability on soils.

To determine sorption of tylosin to clay minerals and desorption, tylosin was adsorbed to three clays (kaolinite, illite, and montmorillonite) in 0.01 M $CaCl_2$ solution. Desorption was carried out by washing tylosin-bound clay minerals with the same solution. The amount of tylosin was quantified by HPLC. The results showed that tylosin was strongly sorbed to clay minerals, reaching maximal adsorption at 24 h. The adsorption to three clay minerals was well described by power function equation ($q_t = at^b$) with $R^2 = 0.95$ to 0.99, and was in the order

monotmorillonite > illite > kaolinite. A proportion of Tylosin can be desorbed from mineral

clays in the order illite > kaolinite > montmorillonite.

In conclusion, this study demonstrated that tylosin were stable under light, 0.01 M $CaCl_2$,

pH 4.5 to 6.0, and 25 ^{o}C for at least 12 days. Sorption characteristics of tylosin by three poultry

litter impacted Louisiana soils can be described by the Freundlich equation.   Both soil pH and

organic matter play an important role in tylosin sorption. Tylosin adsorbed on soils remains

stable, and can be desorbed by 0.01 M $CaCl_2$, and extracted further by methanol. Three clay

minerals had different sorption rates with montmorillonite being the highest and kaolinite the

lowest. The desorption of tylosin from the three clay minerals using 0.01 M $CaCl_2$ was the least

with motmorillonite but the most with illite.

VITA

Zehua Zhou was born in Chongqin, China in 1960. She earned a Bachelor of Science degree in soil science and plant nutrition at Guizhou Agricultural College (now Guizhou University), Guiyang, China in 1986. She started her career as a teaching associate in the same university, and then was promoted to instructor and assistant professor. She came to U.S.A in 2000 with her family and pursued a graduate degree in environmental toxicology at Southern University in 2004. In 2005, she transferred to Louisiana State University pursuing a master's degree under the guidance of Dr. Jim Wang. Her study focused on sorption and desorption characteristics of antibiotic tylosin in Louisiana soils and selected clays.