SORPTION OF VETERINARY ANTIBIOTICS TO WOODCHIPS

by

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Abstract

In the upper Midwest, subsurface tile drainage water is a major contributor of nitrate (NO_3 –N) coming from fertilizers and animal manure. Movement of NO_3 -N through tile drainage into streams is a major concern as it can cause eutrophication and hypoxia conditions, as in the Gulf of Mexico. Denitrifying bioreactors is one of the pollution control strategies to treat contaminated tile drainage water. These bioreactors require four conditions which are: 1) organic carbon source, 2) anaerobic conditions, 3) denitrifying bacteria and 4) influent NO_3-N. This research focuses on investigating fate of veterinary antibiotics in woodchips commonly used in in-situ reactors. Tylosin (TYL) and sulfamethazine (SMZ) are two veterinary antibiotics which are most commonly used in the United States and can be found in tile water after manure is land applied. Partition coefficients of TYL and SMZ on wood were determined by sorption experiments using fresh woodchips and woodchips from an in situ reactor. It was concluded that the woodchips were an effective means to sorb the veterinary antibiotics leached into the tile water after application of animal manure. Linear partition coefficients were calculated and phase distribution relationships were established for both the chemicals. The fresh woodchips gave inconclusive data but predictions could be made by the information determined in the experiments using woodchips from a ten year old woodchip bioreactor. Desorption was also studied and the likelihood of desorption was predicted using the Apparent Hysteresis Index.

Overall, it was found that the old woodchips allowed for quick sorption of both antibiotics. It was also found that SMZ had reversible sorption on old woodchips. Thus, it was concluded that the woodchip bioreactor would not be effective for removal of veterinary antibiotics from tile drainage. More research is required for the fate of TYL and to confirm the conclusion.

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Dedication

I would like to dedicate this thesis to my parents, my brother and sister-in-law, who have been the constant source of encouragement and inspiration in all my academic endeavors.

Chapter 1 - Introduction

1.1 General Introduction

Agricultural production can be improved by subsurface tile drainage systems by draining and maintaining the subsurface water levels of the fertile lands. However, the tile drainage can also have a negative impact on surface and subsurface water quality through contamination by nutrients such as nitrates and organic contaminants (i.e., pesticides and veterinary antibiotics). One of the major pollution concerns is the movement of nitrate (NO_3 -N) through tile drainage into surface streams as it can cause eutrophication and hypoxia conditions as in the Gulf of Mexico. Subsurface tile drainage water is one of the major contributors of NO_3 -N in the upper Midwest (Nangia et al., 2010). Fertilizers (50%) and animal manure (15%) are the major sources of nitrogen in tile water (Goolsby and Battaglin, 2000). To minimize the levels of NO_3-N impact, reduction in the use of nitrogen-based fertilizer to decrease the NO_3-N concentration may not be sufficient (Hunt et al., 2008). Thus treatment of nitrate by different control strategies is required; one of the strategies is treatment of nitrate by in situ denitrifying reactors and denitrifying walls where nitrate is reduced when tile water flows through them under denitrifying conditions (Schipper and Vujdovic-Vukovic, 2000, Jaynes et al., 2008, Greenan et al., 2006). To create the denitrifying conditions, denitrifying walls or in situ reactors are typically constructed with a mixture of organic residues such as woodchips or saw dust, and sand.

In addition to denitrification, the woodchips in bioreactors can also remove organic contaminants such as veterinary pharmaceuticals. Veterinary pharmaceuticals can enter the surface and subsurface water after application of manure on fields. The major adverse effect of these veterinary pharmaceuticals is the development of resistant microorganisms to these

antibiotics which in turn can indirectly impact human health through the ineffective treatment of pathogenic bacteria by the antibiotics (Casewell et al, 2003). Interactions of antibiotics with soil microorganisms include: impact on the degradation or detoxification of the anthropogenic chemicals by soil microorganisms, inhibition of growth of certain bacterial communities, changes in relative abundances of bacterial communities among each other, and development of resistance in bacteria for survival (Kemper, 2008). Although sorption of tylosin (TYL) and sulfamethazine (SMZ) has been studied in soils, there is very less information about their sorption to other solid particles. However, Ilhan et al. (2010) recently reported that SMZ was sorbed more strongly to woodchips than soils with a K_d (where K_d is linear distribution coefficient or the linear partitioning coefficient) of 61 L kg^{-1}.

At present, the fate of nitrate in denitrifying in situ bioreactors or denitrification walls has been evaluated, but the fate of veterinary pharmaceuticals in woodchip reactors is unknown. Several factors such as the type and age of woodchips in the bioreactors, pH conditions, and flow rates through the bioreactors may affect the sorption and degradation of veterinary pharmaceuticals. The overall goal of this study was to understand the sorption of tylosin and sulfamethazine on bioreactor woodchips. Tylosin and sulfamethazine are widely used veterinary antibiotics commonly found in swine manure. The specific objectives of this study were to:

1) investigate the sorption-desorption of TYL and SMZ on woodchips and compare them for various retention times of a woodchip bioreactor;

2) investigate the sorption-desorption of TYL and SMZ on two different types of woodchips: a) fresh and b) older, from a bioreactor installed in 1999; and

3) investigate the sorption of TYL and SMZ on different size of woodchips.

1.2 Report Organization

The report contains five chapters with Chapter 1 providing a broad overview of the issues and the goals and objectives of the study. Chapter 2 consists of the literature review providing information on woodchip bioreactors, removal of nitrate by woodchip bioreactors, environmental concentrations, risks, and sorption and degradation of the selected chemicals. Chapter 3 discusses the methods and materials for batch sorption experiments of the selected chemicals onto woodchips. Chapter 4 provides the results obtained from the experiments and discussions. Finally, Chapter 5 provides the main conclusions of the batch sorption experiments and implications for future research.

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Chapter 2 - LITERATURE REVIEW

2.1 Tile Drainage System and Woodchip Bioreactors

2.1.1 Tile Drainage

In the United States, subsurface drainage tile lines are used as a common agricultural practice for artificial drainage in large areas with high productivity but poorly drained soils (Baker and Johnson, 1981; Kladivko et al., 1991; Buhler et al., 1993). Particularly in the upper parts of the Midwest, over 30% of the soils are underlain by tile drainage networks and a large amount of water is drained through the tile to increase agricultural production and maintain productivity (Randall, 1997). However, the tile drainage system has also been held responsible for increasing transport of large amounts of nitrate-nitrogen (NO_3–N) from the soil matrix into streams and rivers (Hatfield et al., 1998; Randall, 1998; Kanwar et al., 2005). The major concern of NO_3--N pollution is that it can cause eutrophication and hypoxia conditions.

Recently, the expanding zone of hypoxia in the Gulf of Mexico has been related to the increasing NO_3–N loading in the Mississippi River (Nangia et al., 2010). Various studies have also associated non-point source pollution of water bodies with NO_3--N contamination from agricultural areas and have also showed high NO_3—N concentrations in tile drainage water due to higher rate of application of N-fertilizers (Cambardella et al., 1999). Therefore, it is useful to monitor tile effluents for assessing the impact of agricultural management practices on surface and groundwater quality (Hallberg et.al., 1986; Kanwar et al., 1987).

2.1.2 Denitrification and Woodchip Bioreactors

Nitrate is produced from fertilizer N applied on the field. NO_3-N production in soil, and lack of significant plant uptake result in the leaching and movement of NO_3-N into tile drainage networks which eventually reach surface waters (Dinnes et al., 2002). From the Midwest, N export via rivers is a contributing factor to the hypoxia problem in the Gulf of Mexico (Rabalais et al., 1996). In 1980 and 1996 the total annual nitrogen load in the Gulf of Mexico was estimated to be 1,568,000 tonne/yr, out of which 61% was NO_3–N (Goolsby et al., 2001). In Southern Minnesota, Iowa, Illinois, Indiana and Ohio, where tile drainage is a common practice, most of the nitrogen in the Mississippi basin comes from agricultural lands (Goolsby et al., 2001)**.** NO_3–N concentrations leaving the subsurface drains are often higher than the USEPA Maximum Contaminant Level (MCL) of 10 mg/L at most of the times of the year except late summer and early fall (Jaynes et al., 1999; Schilling, 2005).

In Iowa, many communities use river water for their drinking water supply and Des Moines, Iowa operates the world's largest NO_3-N removal system at a cost of $3,000 per day of operation to remove NO_3-N from the river water (Greenan et al., 2009). Estimated nitrogen contribution to the Gulf of Mexico from specific sources in the Mississippi Basin includes 50% fertilizers and 15% animal manure (Goolsby and Battaglin, 2000). Nitrogen based fertilizer usage reduction may not be sufficient to decrease the NO_3-N concentration to minimum level of impact (Hunt et al., 2008). Thus, new management and control strategies are needed. Microbial denitrification is one such process that converts NO_3-N to nitrogen gases (N_2 and N_2O), but very less denitrification occurs in subsurface soils due to limited available organic carbon (Cambardella et al., 1999). Denirification can be achieved by placing a porous organic media such as sawdust or woodchips in the flow path of the tile water (Robertson and Cherry, 1995).

One of the strategies is the treatment of nitrate by in situ denitrifying reactors and denitrifying walls where nitrate is reduced when tile water flows through organic media under denitrifying conditions (Schipper and Vujdovic-Vukovic, 2000; Greenan et al., 2006; Jaynes et al., 2008).

Effective removal of NO_3 from agricultural groundwater was seen as the water passed through a trench filled with a mixture of soil and sawdust (a denitrification wall) to promote denitrification **(**Schipper and Vojvodic-Vukovic 2000). The trench consisted of soil mixed with sawdust (30% sawdust) to provide C for denitrification and reported successful reduction in nitrate concentrations from 5 to 16 mg L^{-1} NO_3–N to 2 mg L^{-1} NO_3–N in 10 days. Blowes et al. (1994) constructed a bioreactor filled with woodchips, tree bark shavings, and compost serving as organic C sources to promote NO_3 removal. The reactor was placed at the end of a tile-line and was effective in reducing concentrations of 3 to 6 mg L^{-1} NO_3–N in the drain water to <0.2 mg L^{-1}. Jaynes et al. (2008) used woodchip-based denitrification walls placed on both sides of a subsurface drainage line to remove nitrate from corn–soybean rotation drainage water and achieved a reduction of NO_3–N from 22 mg L^{-1} to 8.8 mg L^{-1} over 5 years of operation. Two small woodchip bioreactors in Ontario reduced nitrate over 4 years by 32% and 53% in drainage from a corn field and a golf course, respectively (van Driel et al., 2006). A mixture of different carbon sources (woodchips, woodchips amended with soybean oil, cornstalks, and cardboard fibers) and subsurface soil was used by Greenan et al. (2006) as the media for an in situ reactor and reported that after 180 days of incubation, nitrate removal with woodchips was 80.13% and removal with corn stalks was 91.75%. For a longer period with woodchips, removal rate of nitrate was found to be steady indicating that woodchips would be more effective in the field than corn stalks (Greenan et al., 2006).

Bioreactors have been successful in decreasing nitrate concentrations in drainage water and shallow groundwater at various locations throughout the world (Blowes et al., 1994; Robertson and Cherry, 1995; Robertson et al., 2000). A typical in field bioreactor varies in volume depending on the drainage area and is designed to treat 20% of the peak flow rate (USDA-NRCS 2009). Four different bioreactors installed in Hamilton, Hancock, Webster and Greene County, Iowa had a volume range from 5200 ft^3 to approximately 9660 ft^3 and had a drainage treatment area ranging from 40 acres to 60 acres (Bhandari., 2010). A schematic of a woodchip bioreactor installed to remove nitrate from tile drainage is shown in Figure 2.1.

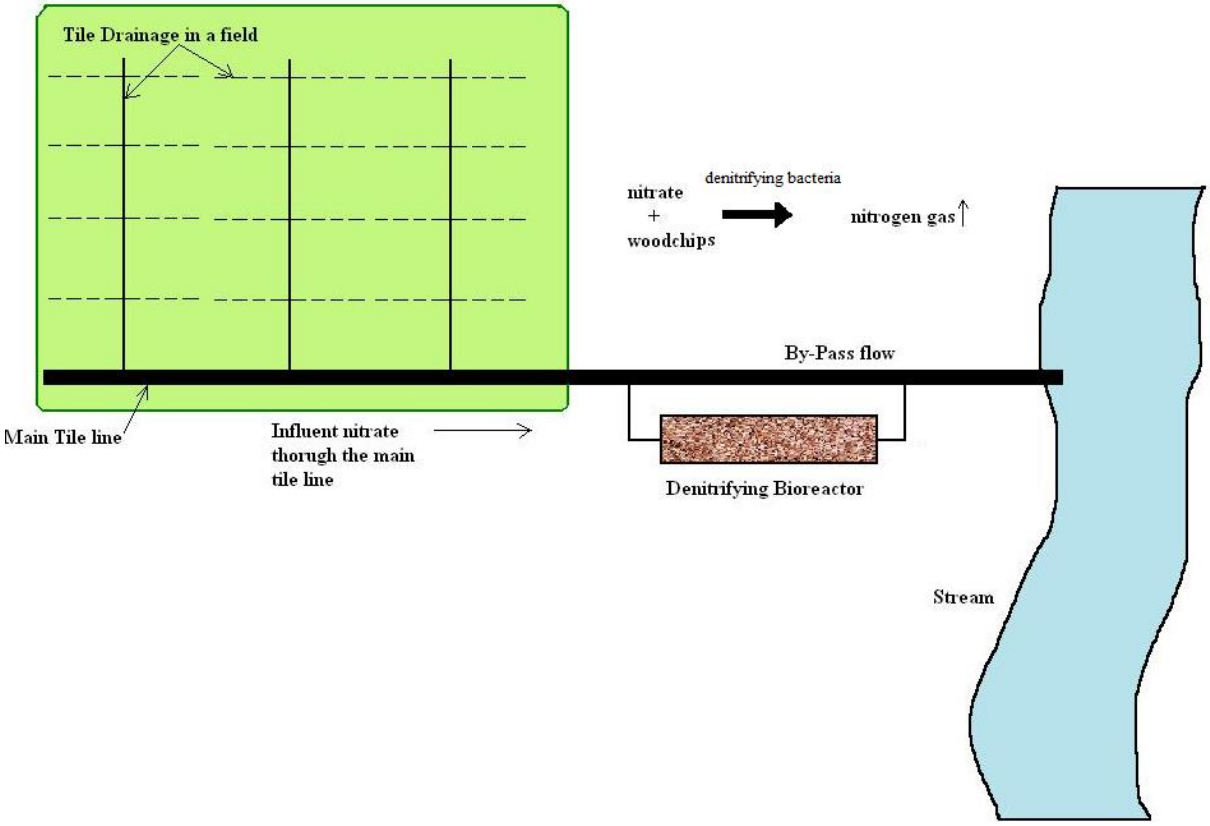


Figure 2-1: Schematic of a Woodchip Bioreactor Installed to Remove Nitrate from Tile Drainage.

The approximate volume of woodchip required can be calculated using the porosity of woodchips. The porosity of woodchips used by Christianson et al. (2010) was 0.67 and that by Van Driel et al., (2006) was reported to be 0.7. Using an average porosity value of 0.7, a reactor volume range of 5200 ft^3 to 9660 ft^3 (Bhandari, 2010), the typical woodchip requirement in a bioreactor would be 1566 ft^3 to 2898 ft^3 (in terms of volume). Christianson et al., (2010) calculated the density for a mixture of local hardwood and found it to be in a range of was 720 to 880 kg/m^3 which was quite similar to density values reported for hardwoods such as oak (820 kg/m^3) (Guyette and Stambaugh, 2003). The amount of woodchips depends on the volume of the bioreactor as per field requirements. However, according to the range of volume of woodchips required and the calculated density, we can calculate an average amount of woodchips required for a typical bioreactor by using an average of 2300 ft^3 woodchip volume and a density of 820 kg/m^3, an amount of 53,300 kg woodchip would be required.

There has been a lot of study on the type of wood used in a bioreactor; Schipper et al. (2010) indicated that there was not much evidence showing a difference in the removal of NO_3-N between hardwoods and softer, quicker growing trees. They also said that both hardwood and softwood have been used in field trial successfully (Schipper et al. (2010).

Retention time of a bioreactor is an important factor to control denitrification. A retention time should be enough to allow bacteria to remove nitrate but not so long to allow any unwanted processes. A short retention time shows less nitrate removal because dissolved oxygen could remain high thus resulting in very less or no denitrification if the system is aerobic. A short retention time may also be too small for the denitrifying bacteria to utilize the nitrate. A longer retention time may result in complete removal of nitrate and other unwanted processes such as production of hydrogen sulfide or methane (Blowes et al. 1994). To manually adjust the retention

time in the reactor, a flow control structure is located near the downstream end of the bioreactor (Christianson et al., 2009).

2.2 Veterinary Antibiotics in the Environment

2.2.1 Consumption Rates and Usage

During 1990s the number of large animal feeding operations for swine, poultry and cattle increased significantly in the United States (USEPA, 2001 in Sarmah et al., 2006). As of 2002 there were approximately 104–110 million cattle, 7.5–8.6 billion chickens, 60–92 million swine, and 275–292 million turkeys in the United States ((AHI, 2002; NASS, 2002) in Sarmah et al., 2006). The use of veterinary antibiotics has become important to the rapidly growing animal food industry. A variety of the veterinary antibiotics have appeared on the market and are widely used in animals to protect from and treat disease, promote growth and improve feed efficiency (Sarmah et al., 2006). Estimates of the total antimicrobial use in North American agriculture are highly variable, ranging from 9 to 16 x 10^6 kg annually with between 10 and 70% being used sub-therapeutically (Sarmah et al., 2006). The potential for rapid spread of disease due to large numbers of animals at concentrated animal feeding operations make the use of pharmaceuticals necessary to maintain the viability of their operations (Sarmah et al, 2006).

Both human and agricultural sources are responsible for the diffusion of antibiotics into the environment. Excretion, flushing of old and out-of-date prescriptions, medical waste, discharge from wastewater treatment facilities, leakage from septic systems and agricultural waste storage systems are few examples. Land application of agricultural waste and wastewater

biosolids, surface runoff and unsaturated zone transport are also few of the major pathways for dissemination of antibiotics. Their environmental impact depends on their physical as well as chemical properties, existing climatic conditions, soil types and variety of other environmental factors. The development of antimicrobial resistant microbial population takes place if antibiotics are not efficiently degraded in the environment (Witte, 1998).

Recent studies have shown that veterinary antibiotics are readily transported into surface water and groundwater from urban and agricultural sources. Thus antibiotic contamination of ground and surface waters is of great concern due to the potential impacts on aquatic and terrestrial ecosystems (Kolpin et al., 2002) and microbial resistance (Sarmah et al., 2006).

2.2.2 Sources and Environment Concentrations

Tylosin is an antibiotic used extensively in swine production for both growth promotion and therapeutic purposes. Typical sources of TYL and its metabolites are through excretion in the swine manure. The manure is treated in lagoons and the lagoon slurries are periodically applied to agricultural fields. TYL residuals may also be applied to fields with manure (Kolz et al., 2005). Kolz et al. (2001) indicated that although TYL has low mobility in soils, the addition of manure to soils can affect its mobility by increasing the organic content of soils.

Tylosin is one of the most common antibiotics present in swine and turkey manures, with concentrations as high as 4 mg/L measured in swine manure (Sassman et al., 2007). The Minimum Inhibitory Concentration (MIC) of Tylosin phosphate is 2-16 µg/ml (MIC is bacteria specific, i.e., it depends on the bacteria (in this case, *Campylobacter*) as to how much

concentration of antibiotic it can take up before it becomes antibiotic resistant (Berrang et.al., 2007).

SMZ is used for therapeutic purposes, for treatment of infections, and as a growth promoter (Tolls, 2001). Sulfonamides are highly water soluble and are mobile through soil. Because of these two characteristics, they pose risks in contaminated groundwater (Batt et al., 2006). A sulfonamide monitoring study in Idaho revealed that wells of nearby confined animal feeding operations were contaminated with SMZ at concentrations of 0.076-0.22 μg L^{-1} (Batt et al., 2006). Kolpin et al. (2002) reported mean concentrations of SMZ of 0.02-0.22 μg L^{-1} in surface waters in a monitoring study of more than 100 rivers**.** Besides surface and subsurface waters, sulfonamides have been detected in soils at concentrations as high as 11 ng g^{-1} (Höper et al., 2002). The MIC of sulfonamides can be as low as 2 μg L^{-1} and can be as high as 515 μg L^{-1} for gram-negative aerobes (Prescott et al., 2000).

2.2.3 Fate and Transport in Groundwater and Environmental Concerns

Many antibiotics used in the animal food-producing industry are poorly adsorbed in the gut of the animal, allowing as much as 30–90% of the antibiotic to be released through excretion (Sarmah et al., 2006). Human-use antibiotics are directly discharged to the environment as a point source and veterinary antibiotics are primarily introduced to the environment as a non-point source. Veterinary antibiotics are generally introduced to agricultural fields in manure applied as fertilizer. They have been detected in soil at high concentrations and can persist for long periods of time (Hamscher et al., 2002), increasing the chance of transport into other environments. After the antibiotics come in contact with the soil, they may be sorbed onto the soil particles or may leach into the groundwater and reach the rivers or streams. Antibiotics can

also enter the environment through manufacturing plants, leakage from waste storage containers and structures, surface runoff, process effluents, and discarding expired or unused compounds (Sarmah et al., 2006). Once in the environment, veterinary antibiotics can have adverse effects. One immediate concern is the development of bacteria that are resistant to the administered veterinary antibiotics. As these compounds fail to fully degrade and remain bioactive in environmental media, they could help develop and maintain antibiotic resistant bacteria (Sarmah et al., 2006).

Available data show that residues may be present in manure and slurry spread on land and veterinary pharmaceuticals may affect terrestrial and aquatic organisms and play a role in the development of antimicrobial resistance (Kay et al. 2003). A limited amount of information is available on concentrations of these compounds in soil (Hamscher et al., 2002; Kay et al., 2003), surface water, and groundwater (Hirsch et al., 1999; Kolpin et al., 2002; Kay et al., 2003). The concern that antibiotics introduced to the environment can result in bacterial resistance is significant as it eventually results in ineffectiveness of the antibiotic in human health management (Kim et al., 2009). A study performed in 2002 on a residential garden treated with manure, a hospital lake, and a dairy farm canal saw bacteria resistance of 70, 75, and 77% respectively (Esiobu, 2002). Drinking water or food including meat and plants fertilized with manure were found to be good media for the resistant bacteria to enter humans (Kümmerer, 2009). Human antibiotics and veterinary antibiotics are often similarly related, and drug effectiveness in humans may decrease if bacteria resistance is developed. Moreover, another source of antibiotic resistance in the food chain may be groundwater (Sarmah et al., 2006). Although veterinary antibiotics are less likely to be found in groundwater than surface water, leaky septic tanks and farm runoff contaminate groundwater sources (Kümmerer, 2009).

Potential environmental risks posed by these compounds have led many countries (USA, Europe, and Canada) to regulate them in a way that environmental effects are minimized. In the USA, most assessments on environmental risk of veterinary antibiotics can be obtained from the US Food and Drug Administration web site (www.fda.gov/cvm/efoi/ea/ea.htm) (Sarmah et al., 2006). Additionally, very few studies have looked at the processes determining the transport of veterinary medicines in the environment. Therefore, it is difficult to fully assess their risk to terrestrial and aquatic ecosystems and the need to address these compounds to ensure minimal adverse environmental impact (Kay et al., 2003)

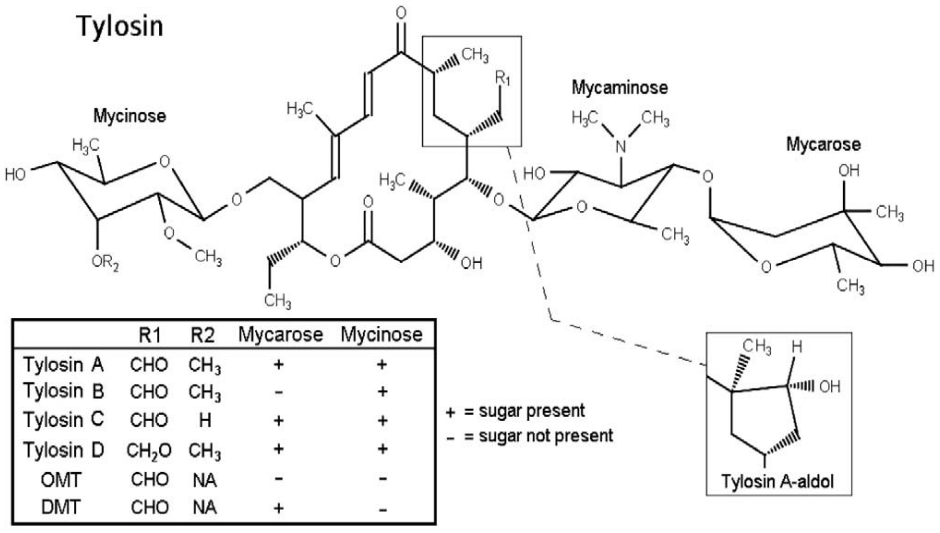
2.2.4 Characteristics of Tylosin and Sulfamethazine

In 1996, TYL was one of the most frequently used antibiotics in swine production with a usage of 30.4% of the total antibiotics used in the United States (Sarmah et al., 2006). Furthermore, Meyer et al. (2003) found that amongst other antibiotics, SMZ was one of the most frequently detected antibiotics at hog animal feeding operations, from six states in the United States.

TYL, which is used on swine, cattle, and chicken for disease prevention, growth promotion, and feed efficiency, is a wide-spectrum, macrolide antibiotic produced by fermentation of *Streptomyces* strains (Sassman et al., 2007). It is a veterinary antibiotic which is comprised of 80-90% Tylosin A and also contains Tylosin B, C, and D macrolides (Sarmah et al., 2006). The molecular structure (Fig. 2.2) of the various components of TYL are characterized by a substituted, 16-membered lactone ring with an amino sugar (mycaminose) attached to the lactone ring at position 5 via a β-glycosidic linkage. Tylosin A, Tylosin C, and Tylosin D all contain mycinose, attached at position 14 of the ring, and mycarose, attached at

position 4 of the mycaminose moiety. The remaining related substances contain either one or neither of these two sugars (Sassman et al., 2007).

With a high solubility, TYL is stable under neutral pH values, and is unstable in acidic or alkaline conditions (Sarmah et al., 2006). Tylosin A and related compounds are stable at pH 4 to 9 (Sassman et al., 2007). Having relatively low mobility, contamination of soil and groundwater is less severe (Sarmah et al., 2006). A recent study in Michigan found TYL was rarely detected in surface water (Song et al., 2010). Kolz et al. (2005) saw a 90% disappearance rate within 5 days of application which shows TYL dissipates quickly degrades when introduced in the environment, although Tylosin B and D were found to be produced as degredates and remained in a slurry lagoon for eight months. In addition, no detection of TYL was noted by Kay et al. (2005) from 2-120 days after an application and found it degraded entirely during slurry storage and/or in the soil. While TYL has low mobility, it has fast and high sorption capabilities to various types of soil with partitioning coefficient (K_d) values between 42-65 L/kg (Hu et al., 2009). Most of the TYL disappeared in a soil column reactor and a batch sorption test which was attributed to sorption in solids (Hu et al., 2009).



	R1	R2	Mycarose	Mycinose
Tylosin A	CHO	CH_3	+	+
Tylosin B	CHO	CH_3	-	+
Tylosin C	CHO	H	+	+
Tylosin D	CH_2O	CH_3	+	+
OMT	CHO	NA	-	-
DMT	CHO	NA	+	-

Figure 2-2 Molecular Structure of Tylosin (Sassman et al., 2007)

After administration to livestock via injection or through feed, TYL is metabolized with Tylosin B, Tylosin D, and dihydrodesmycosin metabolic residues typically found in feces along with the main active ingredient, Tylosin A (Kolz et al., 2005; Sassman et al., 2007). For Tylosin A, half lives of less than 8 days have been reported (Sassman et al., 2007). Tylosin half-lives measured in soils ranged from 6.1 d to three months (Halling et al., 2005; Sassman et al., 2007), and from 17 to 54 d in surface-water samples with a variable lag time of 0 to 60 d (Ingerslev et al., 2001; Sassman et al, 2007). An increasingly stronger or irreversible binding of TYL and metabolites onto manure solids over time may also be the cause of the disappearance of TYL from manure along with microbial and abiotic degradation (Kolz et al., 2005; Sassman et al., 2007). Sorption is expected primarily through cation-exchange interaction for TYL, a weak base with a p*K*a of 7.7 (Sassman et al., 2007). However, because of its large size, some partitioning through hydrophobic forces also may be expected (estimated octanol–water partition coefficient, 43L/L (Sassman et al., 2007)). Few studies have been performed to see the sorption characteristics of TYL to date (Sawhney et al., 1997; Rabolle et al., 2000; Loke et a., 2002; Kumar et al., 2004; Kolz et al., 2005). Rabolle et al. (2000), as well as Kumar et al. (2004), measured sorption of TYL on agricultural soils from Denmark and Minnesota and it was found to be nonlinear and correlated to clay content, with soil–water distribution coefficients (K_d) in the order of 101 to 102.1 L/kg. K_d values for swine manure ranged from 101.6 to 102 L/kg (Loke et a., 2002; Kolz et al., 2005).

Sulfamethazine is used on swine for disease prevention and is typically used with TYL and chlortetracycline (Sarmah et al., 2006). It is part of the sulfonamide group of synthetic and bacteriostatic antibiotics which inhibit bacteria multiplication (Sarmah et al, 2006). Sulfonamides, derived from sulfanilamide are broad-spectrum antimicrobials. They are effective

against both Gram-positive and Gram-negative bacteria including *Chlamydia spp.* (Baroni et al., 2008). Their mode of action is on folic acid biosynthesis in bacteria by competing for dihydropteroate synthetase which interferes with the incorporation of para–aminobenzoic acid (PABA) with the folic (pteroylglutamic) acid. pKa values for sulfonamides vary from 5.0 to 10.4 (Prescott et al., 2000). As shown in Figure 2.3, the essential part of the molecule is the para-NH_2 group with the amide NH_2 group substitutions changing the antimicrobial activity of the compound. Sulfamethazine (4-amino-N-(4,6-dimethylpyrimidin-2-yl) benzenesulfonamide) CAS Number: 57–68–1, molecular weight: 278.33) is the most widely used sulfonamide in animal husbandry (Huang et al., 2001). The water solubility of SMZ is 1500 mg L^{-1} (Lertpaitoonpan et al. 2009).

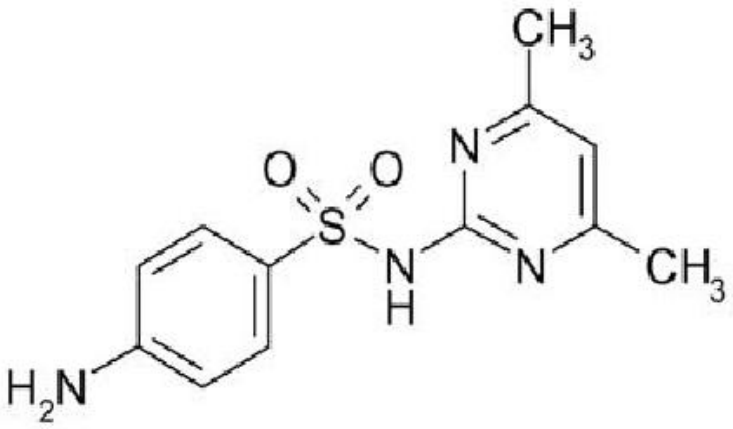


Figure 2-3 Molecular Structure of Sulfamethazine (Babić et al., 2006)

Sulfonamides are easily transported, and their mobility increases with pH values greater than 7.5 (Boxall et al, 2002). SMZ conjugates with sugars in the liver, which activates the compound (Renner, 2002 in Sarmah et al. 2006). As microbes degrade the sugars, the compounds can return to their bioactive forms when excreted. (Renner, 2002 in Sarmah et al., 2006). SMZ remains in the environment for extended periods of time because sulfonamides themselves are not easily biodegradable (Ingerslev; Halling-Sørensen, 2000). SMZ has been detected in soils up to a year after initially applied, even being found in feedlots where it was not administered, showing lengthy persistence time and high mobility in runoff (Aust et al., 2008). An excretion rate of 90% can be seen with SMZ (Aust et al., 2008), and its high mobility made it the second most frequently seen antibiotic in hog liquid waste in animal feeding operations (Meyer et al, 2003 in Sarmah et al., 2006).

2.3 Sorption

2.3.1 Sorption

One of the very important processes affecting the fate of organic compounds such as veterinary antibiotics in the environment is sorption, a process comprised of absorption and adsorption. It plays a major role in deciding the environmental movement, degradation kinetics, and influence of the chemical on environmental processes (Koskinen et al., 1990).

It is common to explain the partitioning of pesticides in soils, typically based on organic carbon content, but it does not apply for all veterinary antibiotics (Kümmerer, 2004). Lertpaitoonpan et al. (2009) found that SMZ sorption onto soils was influenced by organic carbon content of the soil and the pH, with linear sorption coefficients (K_d) of 0.58 and 3.91 L kg^{-1} at pH 5.5 with

organic carbon contents of 0.1% and 3.8%, respectively. When pH was at 9, the K_d values decreased to 0.23 and 1.16 L kg^{-1} for soils with organic carbons of 0.1% and 3.8%, respectively (Lertpaitoonpan et al., 2009). Partition coefficients between soil and solution (K_d) were 0.6 L kg^{-1} for SMZ in a clay loam soil (Thurman et. al, 2000) and 8.3 L kg^{-1} for TYL in a loamy sand soil (Rabolle et al., 2000). In a recent study, Ilhan et al. (2010) studied sorption of SMZ on three different soil depths and K_d for SMZ showed a difference when compared to sorption onto soils of different depth. Depths of 0-15 cm, 80-120 cm and 168 cm had K_d values of 5.5, 9.8 and 0.6 L/Kg respectively. SMZ sorption on woodchips was also conducted and a sorption coefficient of 61 L kg^{-1} was observed (Ilhan et al. 2010).

2.3.2 Sorption Isotherms

In order to predict movement of agrichemicals in soils, sorption coefficients are estimated. Different models are employed to approximate sorption data for soils and sediments. Linear models of the following form are frequently employed:

$$q_e = K_D C_e$$

where q_e is the mass of solute sorbed per unit mass of solid at equilibrium, C_e is the solute concentration of the solution and K_D is linear distribution coefficient or the linear partitioning coefficient. Many conceptual models have suggested this formulation of solute partitioning to organic phases (Weber et al, 1992). The octanol water partitioning coefficient K_{ow} of a solute and the mass fraction of the organic carbon f_{oc} (where foc is the mass fraction of soil organic carbon content are the two readily obtained parameters required to predict sorption behavior for a particular solid.

$$\log(K_D / f_{oc}) = \log(K_{oc}) = a \log K_{ow} + b$$

The coefficients *a* and *b* in correlations of this type are obtained experimentally, but they have been related to sorbent properties (Weber et al, 1992). Lot of evidence exists to show that variations in sorption behavior and differences in K_{oc} (where K_{oc} or the soil organic carbon-water partitioning coefficient is the ratio of the mass of a chemical that is sorbed in the soil per unit mass of organic carbon in the soil at equilibrium) between different soils and sediments can be attributed due to variations in the origin and types of organic matter associated (Weber et al, 1992). But a major constraint on the equations being conceptually correct and can be utilized generally is that only linear equilibrium relationships is assumed and accommodated. However, there is abundant evidence of isotherm nonlinearity in subsurface soils (Weber et al, 1992). For surface adsorption phenomena into organic matrices, non linearity is expected for a range of different solution concentrations, if the sorption affinity increases or decreases significantly with time. Various conceptual and empirical equilibrium models exist for representing nonlinear sorption processes. One of them is the Langmuir model which is predicated on an asymptotic approach to a maximum sorption capacity**,** Q_o and a factor, *b*, which relates to the affinity of the surface for the solute:

$$q_e = Q_o b C_e (1 + b C_e)$$

Several sorption processes are not as energetically straightforward as suggested by either the linear free energy partitioning model or the homogeneous surface and constant energy model. Equilibrium data are typically best described by models which do not assume either homogeneous sorption sites or limited levels of sorption, such as the Freundlich model:

$$q_e = K_F C_e^{\,n}$$

The parameter K_F in equation is termed the Freundlich unit-capacity coefficient or the Freundlich capacity parameter, and *n* is the linearity parameter. Sorption data is plotted as phase distribution relationships (PDRs). A PDR relates the solid phase concentration of a target chemical, q(t) to its aqueous phase concentration C(t), under non-equilibrium conditions (Weber Jr. et al., 1996). PDRs transform into a sorption isotherm when equilibrium is attained.

2.3.3 Sorption of Organics on Wood

Although sorption of TYL and SMZ has been studied in soils, there is very less information about their sorption to other solid particles. Effect of organic matter in the adsorption of hydrophobic organic compounds by natural soils or soils amended with OM have been indicated by various studies. Wood, a material of plant origin, contains a series of organic compounds (cellulose, lignin, hemicellulose, resin, tannin, etc.). The presence of high proportions of lignin in wood could make this latter material a good adsorbent of hydrophobic organic compounds, as is the case of many pesticides used in agriculture. Sharma et al. (2008) found that about 74.7 to 80.5% of atrazine was removed by sorption onto sawdust (42.3% C) which was treated with 0.1 N H_2SO_4 and kept at 200°C for 4 hours. Ilhan et al. (2010) conducted batch experiments for sorption of various organic contaminants onto soil as well as woodchips and the sorption coefficients were estimated. K_d values for SMZ, atrazine, enrofloxacin and monesin on woodchips were found to be 61, 24.1, 282 and 24.2 L kg^{-1} respectively. However, in a recent study, Ilhan et al., (2010) showed that SMZ was sorbed more strongly to woodchips than soils as the K_d for SMZ on different depths of soil ranged from 0.6 to 9.8 L kg^{-1} while K_d for SMZ on woodchips was 61 L kg^{-1}**.** Sorption of toluene, benzene and o-xylene on wood was

studied by Mackay et al. (2000) and it was observed that these chemicals reached equilibrium within 24 hours of contact with wood. Sorption to 10 organic compounds to wood was studied by Trapp et al., 2001 and they observed the wood can serve as "safe sink" for environmental chemicals. Thus woodchips, such as the ones used in field bioreactors, can act as a potential sink for antibiotics. As we know, field tiles which are used to drain excess water from agricultural soils in the Midwestern United States, particularly Iowa, transport nitrates and other contaminants like veterinary antibiotics and bacteria. Woodchip bioreactors can serve as a sink for antibiotics in tile drainage through sorption.

2.4. Desorption

2.4.1 Desorption Isotherm and Desorption Hysteresis

Desorption plays a key role in determining the distribution and migration of a contaminant in the environment. A lot of factors affect desorption: Bhandari and Lesan (2003), reported that sorption on soil components, uptake by plants, transport via runoff and leaching, biodegradation, volatilization and chemical degradation could be such factors deciding the fate of atrazine in the soil environment. They also said that one major factor affecting sorption and desorption is natural organic matter whether in soil, sediments, or in solution or in our case woodchips. Various studies have shown limited desorption of sorbed chemicals. Also, the rate and extent of desorption has been observed to decrease with increase in contact time of solid and liquid phase (Weber Jr., 1997). Liwang et. al (1993) studied adsorption and desorption of atrazine onto soil and concluded that the amount of atrazine desorption was a function of incubation time. A simplified approach was established to quantify hysteresis and they defined hysteresis as the

difference between sorption and desorption isotherms. Hysteresis was quantified based on the maximum differences between an adsorption isotherm and a desorption isotherm using the following equation:

$$\omega = \frac{Max(S_d - S_a)}{S_a} \bullet 100$$

where ω is a measure of hysteresis in percent and S_d and S_a are amounts of atrazine sorbed based on desorption and adsorption respectively.

In a study of phenanthrene sorption and desorption, the apparent sorption-desorption hysteresis was quantified using a Hysteresis Index (HI) as described in the following equation:

$$HI = \frac{q_e{}^d - q_e{}^s}{q_e{}^s} \Big| T, C_o$$

where $q_e{}^s$ and $q_e{}^d$ are solid-phase concentrations for sorption and desorption respectively, and T and C_e represent conditions of constant temperature and aqueous phase concentration of atrazine (Huang et al., 1997) Similar to the above study, the effect of contact time on desorption of atrazine was investigated by Bhandari and Lesan (2003). A time dependant Apparent Hysteresis Index (AHI) was used to describe the amount of desorption, where an AHI value of zero indicated no hysteresis. AHI(t) was calculated using the following equation:

$$AHI(t) = \frac{q(t) - q^d(t)}{q(t)} \Bigg|_{C(t)=C^d(t),T}$$

where q(t) and $q^d(t)$ were solid phase atrazine concentrations from sorption and desorption PDRs and C(t) and $C^d(t)$ are corresponding predetermined aqueous atrazine concentration (Bhandari and Lesan, 2003).

2.5. Summary

Leaching of the chemicals and other contaminants from agricultural lands leads to increase in concentration of nitrate and veterinary pharmaceuticals in surface waters and subsurface waters. The amount of water drained from agricultural lands with artificial systems such as tile drainage is typically higher than from lands without tile drainage which also contributes towards nitrate pollution of surface waters, especially in the Midwest. In denitrifying bioreactors, nitrates are denitrified using woodchips or saw dust in the bioreactors as a source of organic carbon, thereby helping in reducing the nitrogen pollution. Along with nutrients, the possible presence of agrochemicals, veterinary pharmaceuticals, bacteria and other contaminants in the tile drainage can also create human health risks and ecological impacts. The fate of nutrients, sediments and agrichemicals in soil have been investigated and documented and the sorption veterinary antibiotic on soil has been studied as well. TYL and SMZ are two widely used veterinary antibiotics in the United States and sorption of these antibiotics would assist in reducing their concentration in subsurface water. However, of interest here is the sorption of these veterinary antibiotics in woodchip bioreactors. Not much is known about the impact of veterinary antibiotics on the denitrifying communities in woodchip bioreactors. Thus, considerable amount of research is required to study the process of partition of veterinary antibiotics onto woodchips.

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Chapter 3 - Methods and Materials

3.1 Chemicals

Sulfamethazine CAS Number: 57–68–1, molecular weight: 278.33, (99% purity) and Tylosin Tartrate CAS Number: 007461055 were purchased from Sigma–Aldrich (St. Louis, MO). Selected physical and chemical properties are presented in Table 3.1 Stock solution of TYL (1000 mg L^{-1}) was prepared in high performance liquid chromatography (HPLC) grade water. Stock solution for SMZ (1000 mg L^{-1}) was prepared in analytical grade acetonitrile. All standards for HPLC calibration curves were prepared by diluting stock solutions in HPLC grade water and were stored at 4 °C under dark conditions.

3.2 Sorbents

Woodchips were used to compare differences in sorption rates and extents. Fresh, new woodchips were obtained from Golden Valley Hardscapes LLC in Story City, IA. These fresh woodchips were the same as used by Christianson et al. (2010) for their pilot bioreactor study which was a number of local hardwood species mixed together and similar to those used in-field bioreactors. Old woodchips were obtained from a field bioreactor, installed in September of 1999 (Moorman et al., 2010) in Boone County, IA, northwest of Ames. The bioreactor was in use from 1999 to 2009 and the woodchips were taken at approximately 120 cm depth below the surface. The woodchips were kept in a sealed bag at 4°C until they were dried in the oven at 70°C and were manually cut to an approximate average size of 2.5x1.0 cm. Percent

moisture content was also calculated for both kinds of wood and was found to be 8.32% and 25.47% for fresh wood and old wood respectively.

Table 3-1 Selected Physical and Chemical Properties of Tylosin and Sulfamethazine

Properties	**Tylosin**	**Sulfamethazine**
General Category	Macrolides	Sulfonamides
Water Quality Standard, Advisory, Recommendation	Minimum Inhibitory Concentration (Bacteria Specific): 2-16 µg/ml of tylosin phosphate (for Campylobacter).	Minimum Inhibitory Concentration (bacteria specific) : 512 µg/ml of SMZ (for E. coli , enterococcus)
Effects	Campylobacter gives rise to Campylobacteriosis (diarrhea, cramping, abdominal pain, and fever within two to five days after exposure to the organism).	Enterococcus and e. coli affects the intestine.
Environmental Concentrations	Campagnolo et. al. (2002) reported concentrations of antimicrobials such as tetracyclines, sulfamethazine ranging from 2.5 to 240 µg/L in eight swine lagoons in Iowa and Ohio.	Kolz et. al., (2001) reported the aqueous concentration of tylosin expected in lagoons to be between 1 to 30 mg/l
Sorption partition coefficient (on soil)	K_d: 8.3 L kg^{-1} to 240 L kg^{-1}(Kolz et al., 2005)	K_d : 0.6 L kg^{-1} to 9.8 L kg^{-1} (Ilhant et al., 2010)
Solubility	5000 mg L^{-1} (25°C water) (Lewicki., 2006)	1500 mg L^{-1} (29°C water) (Lertpaitoonpan et al. 2009)

Molecular formula	$C_{46}H_{77}NO_{17}$ (Lewicki., 2006)	$C_{12}H_{14}N_4O_2S$ (Huang et al., 2001)
Melting Point	128-132 °C (Lewicki., 2006)	197 °C (Material Safety Data Sheet-Sigma Aldrich)
Molecular Wt.	916.1 gm (Lewicki., 2006)	278.33 (Huang et al., 2001)
CAS No.	1401-69-0 (Lewicki., 2006)	57-68-1 (Huang et al., 2001)

3.3 Sorption Experiments at Different Contact Times

Sorption experiments of the two antibiotics onto woodchips were performed in order to compare the extent and behavior of sorption. Sorption-desorption isotherms were generated. For the initial trial, a sorption experiment was conducted in the Water Quality Research Lab (WQRL) at Iowa State University. A total of 25g of old woodchips or new woodchips were transferred into 120-mL glass serum bottles. The bottles were filled with synthetic tile water solution containing both antibiotics (TYL and SMZ) at initial concentrations of 10 mg L^{-1} each. The synthetic tile water consisted of 350 mg L^{-1} calcium chloride to achieve an ionic conductivity approximately equal to 765 µS which is similar to that of tile water. Preliminary experiments on sorption of SMZ and TYL on wood were observed for 7 day contact time and the results were used for the method development. Sorption of SMZ resulted in a loss of SMZ of upto 97% on old woodchips and 56% on new woodchips; the loss of TYL was upto 81% on old woodchips and 44% on new

woodchips (Figures 3.1 and 3.2). A loss of 97% is a desirable removal rate in a field setting, but 20-80% loss is more desirable while conducting a sorption experiment as it ensures data are within a measurable range.

Sorption experiments were conducted using 90 glass serum bottles with a volume of 120mL each. The bottles were weighed when empty; 30 bottles were filled with approximately 25g of new woodchips, 30 bottles were filled with approximately 20g of old woodchips and 30 contained no woodchips to serve as controls. Each bottle containing woodchips was weighed and then rinsed with high purity water several times by shaking them on an orbital shaker between washes to remove soluble organics and color released by the woodchips. The woodchips were rinsed thoroughly to minimize interference with the HPLC analysis. The bottles were drained, refilled, and shaken approximately 20-25 times until the drained liquid was nearly clear.

After washing the woodchips in the bottles, the bottles were weighed again to account for the mass of water in the woodchips. Solutions containing both TYL and SMZ in concentrations of 1, 3, 5, 8, and 10 mg/L were prepared in synthetic tile water. Triplicates of the new woodchips, old woodchips and controls were used for each concentration of antibiotics and were filled with solution with no headspace. The exact volume of solution added was calculated using the weight of the solution in the bottles after the antibiotics were added. Teflon lined silicone caps were used to close the bottles and aluminum crimp caps were used to seal them. The bottles were then placed on the orbital shaker (Figure 3.3c) at 1500 RPM continuously. Samples were taken from the same bottle at 3, 6, and 12 hour contact times. This was repeated with triplicate bottles for 1, 3, and 7 day contact times and 500µL samples were collected in 2mL vials for HPLC analysis.

Fig 3.1(a)

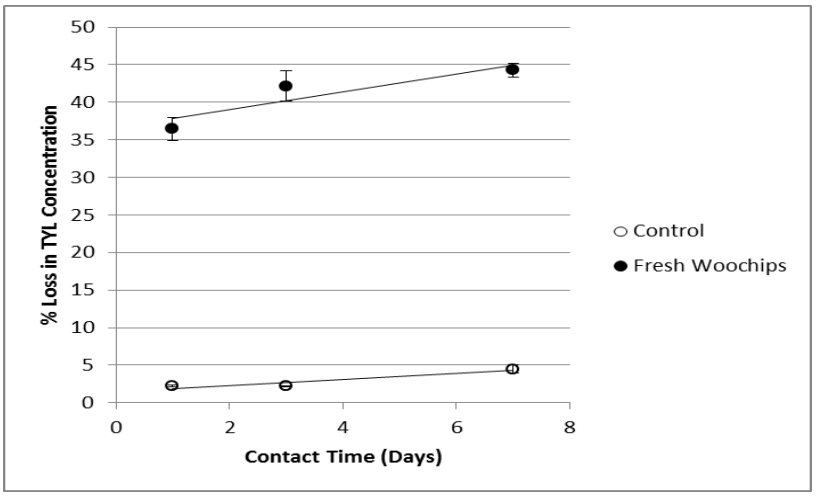

Fig 3.1 (b)

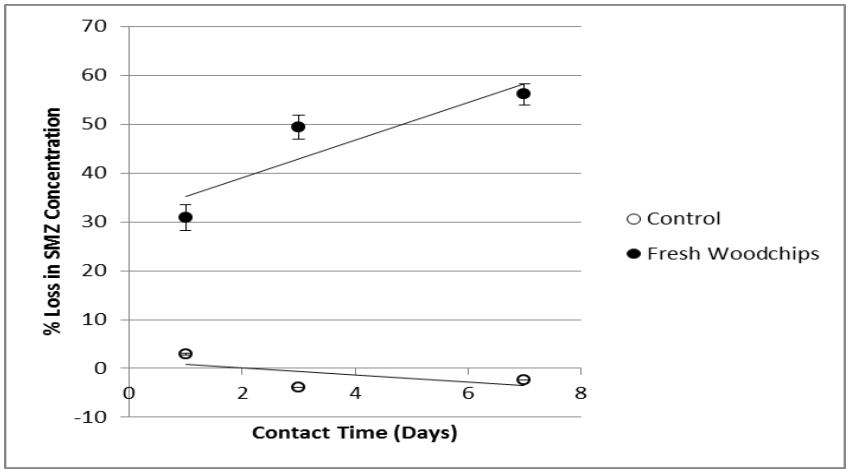

Figure 3-1: Loss of Tylosin and Sulfamethazine due to Sorption on Fresh Woodchips (Solid to Liquid ratio: 25 gm in 70 ml.)

Note: Error bars represent the standard deviation of a data point from mean, calculated from triplicates. It denotes the interval in which the mean of data lies.

Fig 3.2 (a)

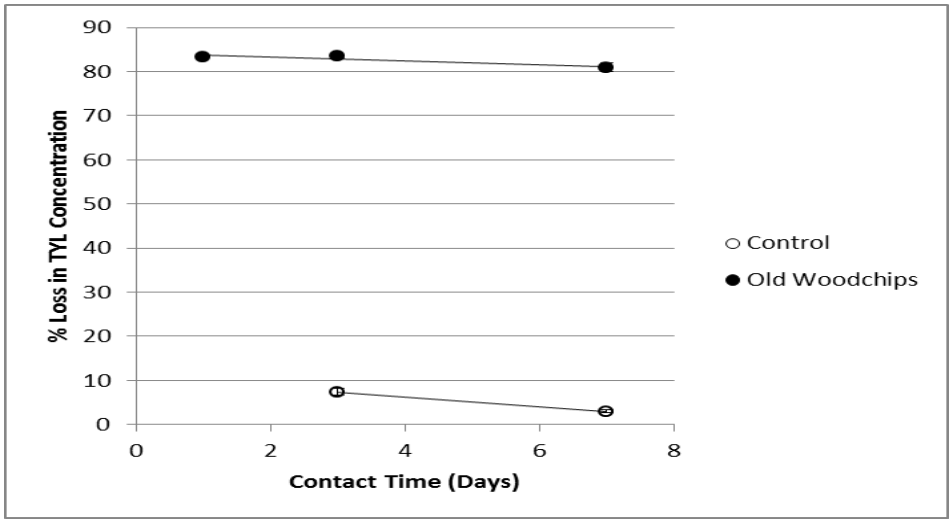

Fig 3.2 (b)

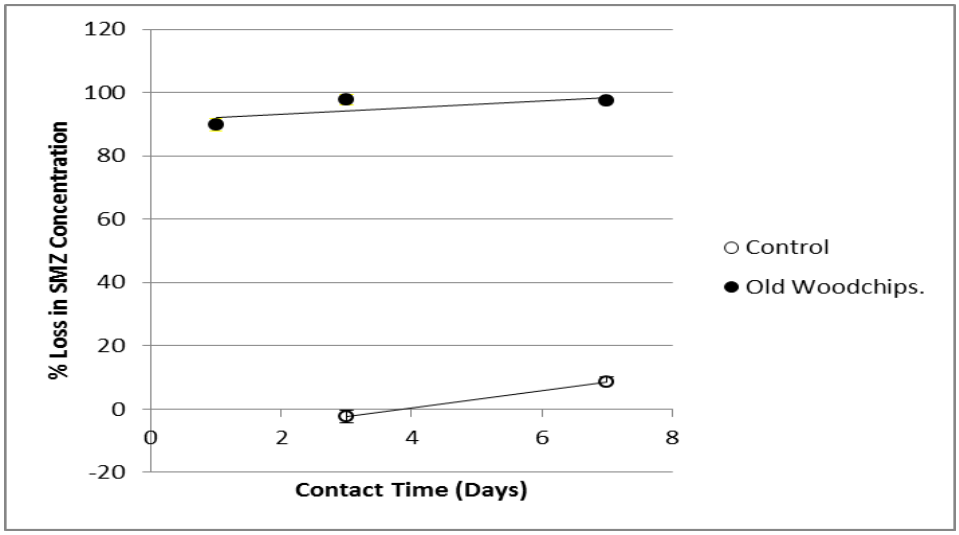

Figure 3-2: Loss of Tylosin and Sulfamethazine due to Sorption on Old Woodchips (Solid to Liquid ratio: 25 gm in 70 ml.)

Note: Error bars represent the standard deviation of a data point from mean, calculated from triplicates. It denotes the interval in which the mean of data lies.

The solid-phase concentrations (mg/kg) were calculated on the basis of the difference between initial aqueous phase concentration and equilibrium aqueous phase concentration of the

chemical. Any losses to air or glass or during sampling were accounted for in the initial phase concentration by using the control concentration. Sorption-desorption isotherms were determined by fitting the aqueous phase concentration versus the solid phase concentration. Partition coefficient (K_d) was calculated using the following equation:

$$q_e = K_D C_e$$

where q_e and C_e are solid phase and aqueous phase concentrations of the chemical at equilibrium. Samples were analyzed against controls using a high-performance liquid chromatogram (HPLC) with a photo diode array detector. Standard curves for TYL and SMZ were determined. Standard curves ranged from concentrations of 0.5, 1.0, 2.5, 5.0, and 10.0 mg/L using manually made standards and after confirming the consistency of the instrument. Data were compiled in Microsoft Excel and phase distribution relationships (PDR) and K_d equilibrium values were determined.

3.4 Woodchip Particle Size for Sorption Experiments

An experiment was conducted to assess the effect of woodchip particle size on TYL and SMZ sorption. The woodchips were manually cut into three different sizes i) original size; ii) medium size approximately 4.0x1.0 cm and iii) fine size: 0.5 x 0.5 cm. A total of 25 gm or 20 gm of new or old woodchips respectively were transferred in triplicates into 120 mL serum bottles. A concentration of 10 mg L^{-1}, each of TYL and SMZ was used. Bottles containing no woodchips were used as controls. The test was conducted for 7 days. Teflon caps were used to close the bottles and aluminum crimp caps were used to seal them. The bottles were then placed on the

orbital shaker at 1500 RPM continuously. This was repeated with triplicate bottles for 1, 3, and 7 day contact times and 500µL samples were collected in 2mL vials for HPLC analysis.

Another woodchip particle size experiment was conducted in the Environmental Engineering Research Lab at Kansas State University, The woodchips were separated into 3 different sizes manually cutting them to an approximate average size of 4.0x1.0cm and fine particle sizes of approximately 0.5x0.5 cm. A total of 27gm of either new or old woodchips were added in triplicates 160mL serum bottles. A concentration of 10 mgL^{-1} each of both TYL and SMZ was used. Bottles containing no woodchips were used as controls. The test was conducted for 10 days. Teflon caps were used to close the bottles and aluminum crimp caps were used to seal them. The bottles were then placed on the orbital shaker at 1500 RPM continuously. This was repeated with triplicate bottles for 1, 3, 7 and 10 day contact times and 500µL samples were collected in 2mL vials for HPLC analysis.

3.5 Desorption Experiments

Desorption was done for 24 hours after completing the 7 day sorption experiments. Fresh water was added in the batch reactors. The bottles were placed on an orbital shaker at 1500RPM, 500µL samples were collected in 2 mL vials for HPLC analysis. Time dependant Apparent Hysteresis Index (AHI) was used to describe the amount of desorption, where an AHI value of zero indicated no hysteresis. AHI(t) was calculated using the following equation:

$$AHI(t) = \left. \frac{q(t) - q^{d}(t)}{q(t)} \right|_{C(t)=C^{d}(t),T}$$

where q(t) and $q^d(t)$ were solid phase atrazine concentrations from sorption and desorption PDRs and C(t) and $C^d(t)$ are corresponding predetermined aqueous atrazine concentrations (Bhandari and Lesan, 2003).

3.6 Chemical Analysis

TYL and SMZ in aqueous solution were analyzed simultaneously using a Varian HPLC Prostar Series (Figure 3.3d) with photo diode array detection. The HPLC eluent flow rate was set at 0.3 mL min^{-1} with the following solvents and times: 0.01 to 1 minute with 10% acetonitrile and 90% water (with 0.01 M ammonium acetate and pH adjusted to 4.5 by glacial acetic acid) followed by increasing the acetonitrile to 100% till 17 minutes. Phenol was tried as internal standard for the analysis but it gave inconsistent data, thus no internal standard was used. Retention times for SMZ and TYL were approximately 9 and 13 minutes, respectively. Detection wavelengths for SMZ were 270nm while the wavelength for TYL was 290 nm. HPLC column temperature was set at 40 °C. The HPLC was equipped with Varian C-18 column 150 x 2.0 mm based on the method developed by Kolz et al., (2001) with modifications. The injection volume was set at 100 µL.

3.7 Statistical Analysis

To test the linearity of K_d for TYL and SMZ with time, regression analysis was performed using SAS (Statistical Analysis Software). Regression analysis was performed for both sorption and desorption isotherms using SAS (Statistical Analysis Software) to test whether there is reversible

sorption. Paired t-test was used to determine equilibrium point for K_d. For the loss percent of SMZ and TYL, error bars with standard deviations were computed using excel.

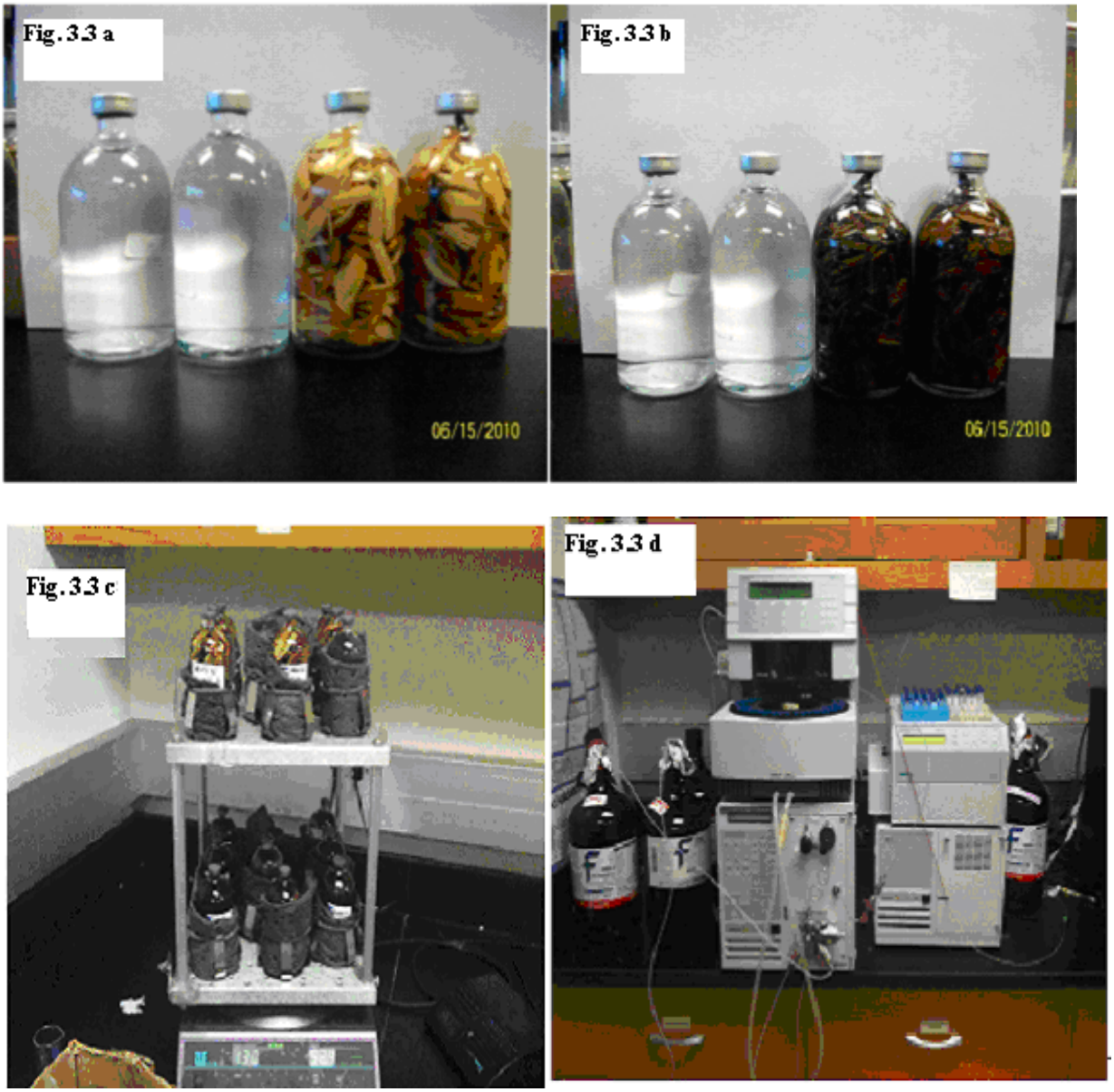

Figure 3-3: (a) Batch Reactor with Fresh Woodchips, (b) Batch Reactor with Old Woodchips, (c) Bottles Placed on the Orbital Shaker (d) HPLC Setup

Chapter 4 - Results and Discussion

4.1 Sorption Study on Woodchips

Chromatograms showed the SMZ peaks around 9 min. and TYL peaks around 13 min. The controls showed smooth baselines and clear peaks (Figure 4.1). Chromatograms of the antibiotics for sorption on old woodchips also had relatively smooth baselines and clear peaks in accordance with the controls (Figure 4.2). However, chromatograms for sorption on new woodchips did not show regular baseline and a second peak was observed just around the SMZ peak (Figure 4.3). This was inconsistent with the chromatograms for sorption on old woodchips and controls. Also, it was sometimes difficult to distinguish the two peaks and accurately integrate the area under the curve because of the second peak. Thus, data for the new woodchips showed no predictable trends and was not used to develop sorption isotherms.

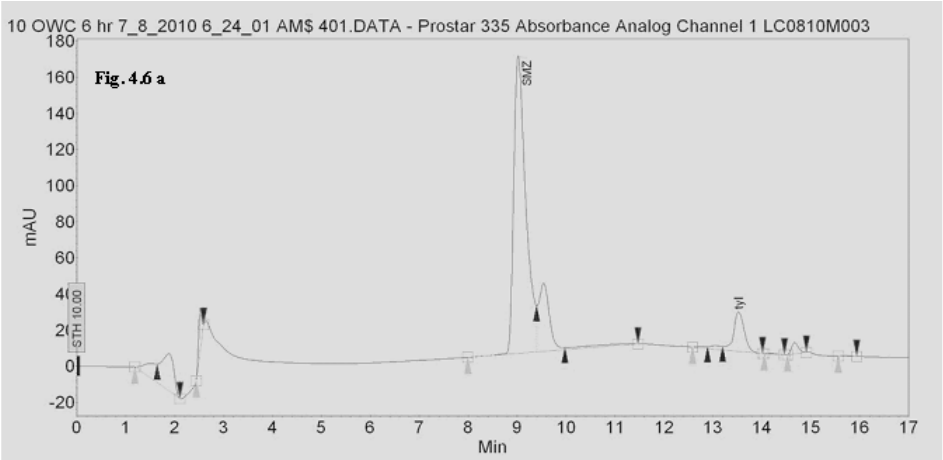

Figure 4-1: Sample High Pressure Liquid Chromatography (HPLC) Chromatogram of a Control Bottle at Concentration of 10 mg/L of both Tylosin and Sulfamethazine and a Contact Time of 6 hrs

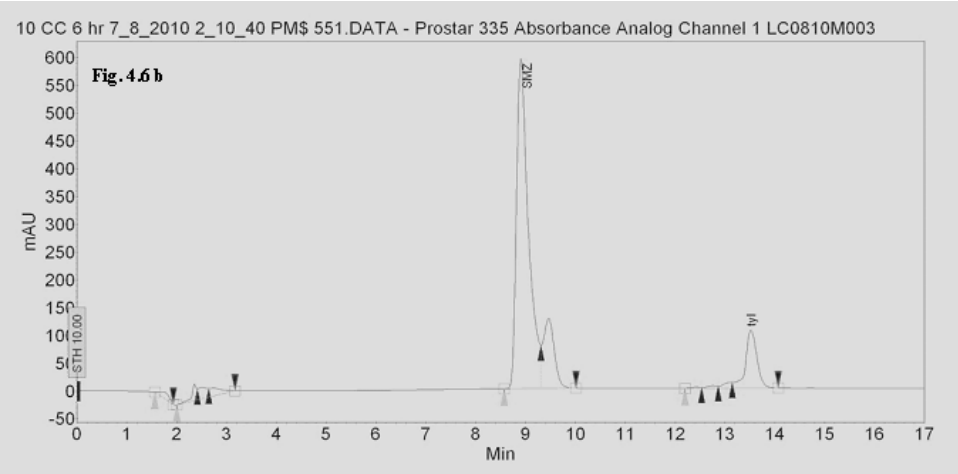

Figure 4-2: Sample High Pressure Liquid Chromatography (HPLC) Chromatogram of a Bottle with Old Woodchips at Concentration of 10 mg/L of both Tylosin and Sulfamethazine and a Contact Time of 6 hrs

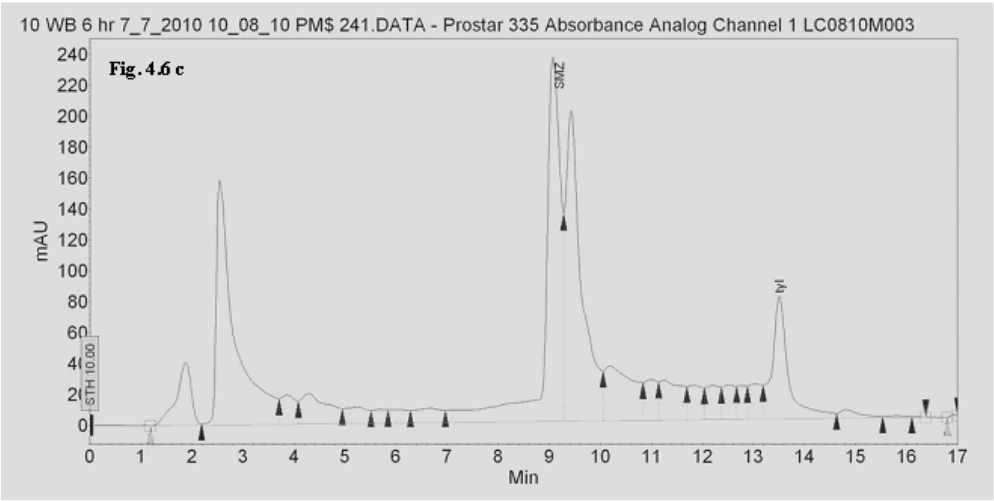

Figure 4-3: Sample High Pressure Liquid Chromatography (HPLC) Chromatogram of a Bottle with New Woodchips at Concentration of 10 mg/L of both Tylosin and Sulfamethazine and a Contact Time of 6 hrs

A solid to liquid phase distribution curve was developed by plotting C(t), the concentration of the antibiotics in the liquid, against q(t), the concentration of the antibiotics in the solid phase. In general, for the old woodchips, both antibiotics showed an increased slope for increased contact

time in their respective PDRs indicating an increase in solid phase concentration with time (Figures 4.4 and 4.5) Figures 4.6 and 4.7 for TYL. As seen in the PDRs the error is quite high and thus exact prediction of sorption cannot be made. The K_d and R^2 values are shown in Table 4.1.

To test the K_d for TYL, regression analysis was performed using SAS (Statistical Analysis Software). The regression coefficient was found to be different from zero (Estimate = 0.014, P value =0.057). Thus it shows that K_d value increases with time for the contact period of 7 days. For TYL, the K_d value goes down to a significant amount and then rises to 6.2 L Kg^{-1} which makes it difficult to conclude whether equilibrium was achieved after 7 days of contact period. It can be seen in Figure 4.8 (a) that TYL seems to reach equilibrium but it is difficult to predict the contact time as the 12 hour data is almost similar to the 72 and 168 hour data. A paired t- test was performed to confirm this hypothesis. Assuming a null hypothesis that the Kd at 12 hour is not equal to the K_d at 168 hours ($\mu_1 \neq \mu_2$) and alternative hypothesis to be ($\mu_1 = \mu_2$), a t-test was performed on the dataset to determine equilibrium point for K_d. A P-value of 0.1771 suggests rejection of null hypothesis thereby indicating that equilibrium was attained at time t = 12 hrs. The error bars indicate that the value of K_d could lie between 5.1 ± 2.29. To test the K_d for SMZ, regression analysis was performed using SAS (Statistical Analysis Software). The regression coefficient was found to be different from zero (Estimate = 0.03, P value =0.0009). Thus it shows that K_d value increases with time for the contact period of 7 days. It can be seen in Figure 4.8 (b) that SMZ does not seem to reach equilibrium. A paired t-test was performed to confirm this hypothesis. Assuming a null hypothesis that the K_d at 72 hour is not equal to the K_d at 168 hours ($\mu_1 \neq \mu_2$) and alternative hypothesis to be ($\mu_1 = \mu_2$), a t-test was performed on the dataset to

determine equilibrium point for K_d. A P-value of 0.0029 suggests that null hypothesis cannot be rejected thereby indicating that K_d is ncreasing with time and did not reach equilibrium.

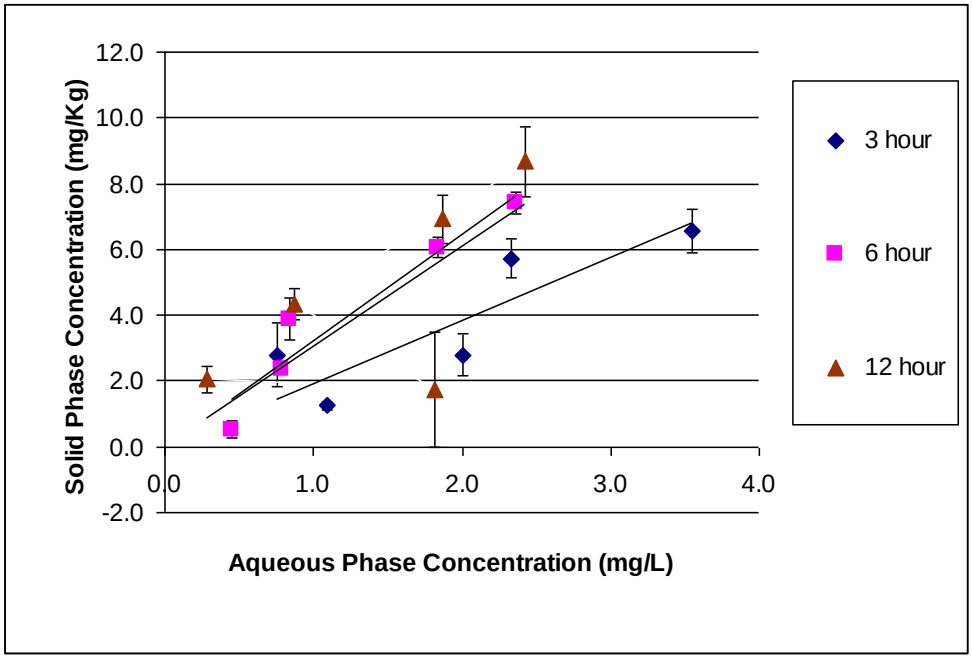

Figure 4-4: Phase Distribution Relationships of Sulfamethazine on Old Woodchips for 3, 6 and 12 Hour Contact Times (Solid to Liquid Ratio: 25 gm in 70 mL)

Note: Error bars represent the standard deviation of a data point from mean, calculated from triplicates. It denotes the interval in which the mean of data lies.

Preliminary results for sorption of TYL and SMZ were used for method development and experiments were conducted to estimate the sorption coefficients for the sorption of TYL and SMZ on old woodchips in batch sorption experiments. The initial aqueous phase TYL and SMZ concentrations in the sorption experiment with old woodchips were 1, 3, 5, 8 and 10 mg L^{-1}. Concentrations used in this experiment are several magnitudes greater than those typically found in soils or water (Cessna et al., 2010) but the assumption of a linear sorption relationship can

allow prediction of phase distribution at lower concentration also. The K_d values for each contact time were plotted. Statistical analysis suggested that K_d both TYL and SMZ increased with time.

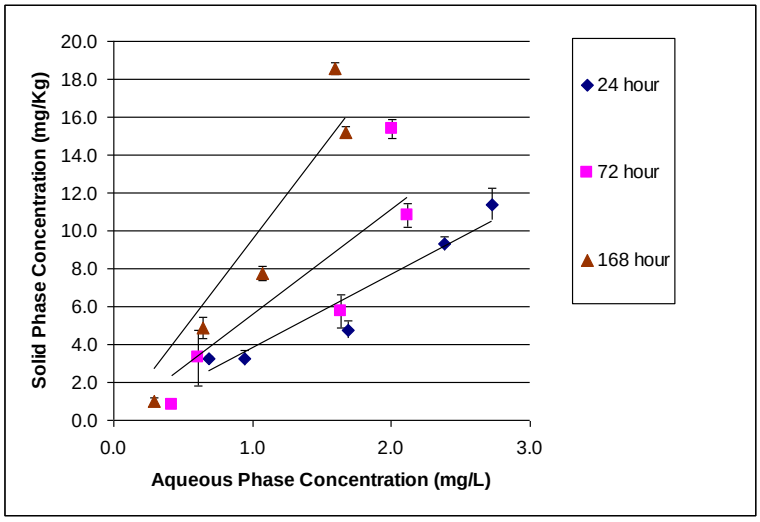

Figure 4-5: Phase Distribution Relationships of Sulfamethazine on Old Woodchips for 24, 72 and 168 Hour Contact Times (Solid to Liquid Ratio: 25 gm in 70 mL)

Note: Error bars represent the standard deviation of a data point from mean, calculated from triplicates. It denotes the interval in which the mean of data lies.

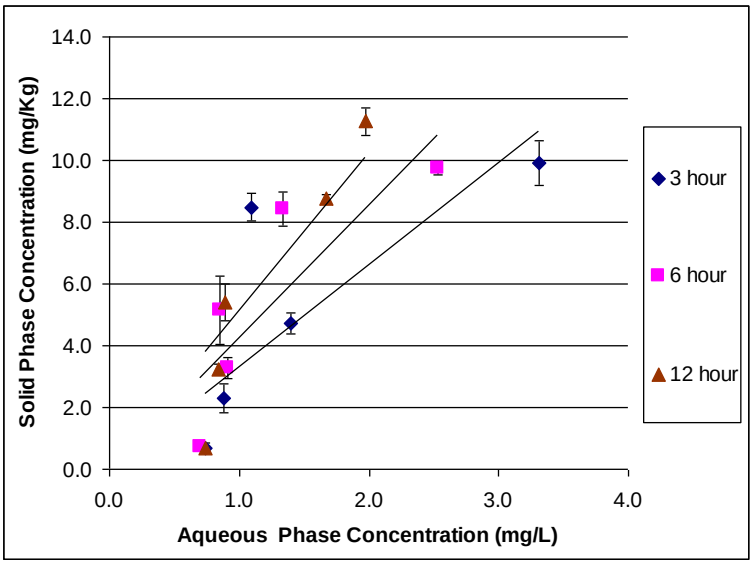

Figure 4-6: Phase Distribution Relationships of Tylosin on Old Woodchips for 3, 6 and 12 Hour Contact Times (Solid to Liquid Ratio: 25 gm in 70 mL)

Note: Error bars represent the standard deviation of a data point from mean, calculated from triplicates. It denotes the interval in which the mean of data lies.

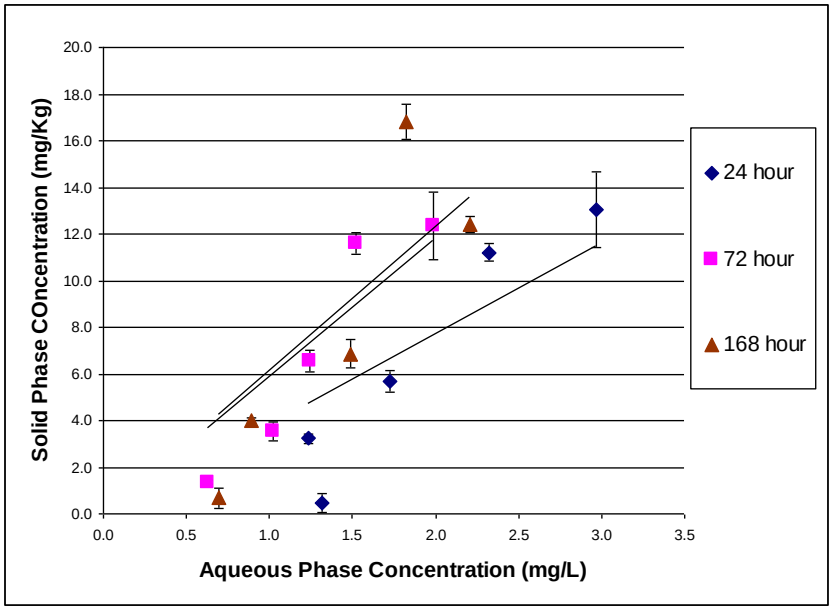

Figure 4-7: Phase Distribution Relationships of Tylosin on Old Woodchips for 24, 72 and 168 Hour Contac Times (Solid to Liquid Ratio: 25 gm in 70 mL)

Note: Error bars represent the standard deviation of a data point from mean, calculated from triplicates. It denotes the interval in which the mean of data lies.

Table 4-1: Linear Partition Coefficients for Tylosin and Sulfamethazine for Sorption on Old Woodchips

Hours	K_d (TYL)	R^2	K_d (SMZ)	R^2
3	3.3 ± 1.03	0.55	1.9 ± 0.73	0.73
6	4.3 ± 1.25	0.70	3.2 ± 1.86	0.92
12	5.1 ± 2.29	0.82	3.1 ± 1.38	0.4
24	3.9 ± 2.54	0.71	3.8 ± 1.77	0.92
72	5.9 ± 2.13	0.79	4.9 ± 2.17	0.78
168	6.2 ± 2.09	0.68	7.8 ± 2.95	0.90

Note: Error bars represent the standard deviation of a data point from mean, calculated from triplicates. It denotes the interval in which the mean of data lies.

The last observed value of K_d was 6.2 L kg^{-1} and 7.8 L kg^{-1} at 7 days respectively (Figures 4.8 a and 4.8 b). Although, the equilibrium K_d for SMZ could not be determined, it can be assumed that K_d would be greater than 7.8 L kg^{-1} i.e. the K_d value for the 7 day contact time. SMZ sorption on woodchips was also conducted by Ilhan et al. (2010) for a contact time of 48 hours and a sorption coefficient of 61 L kg^{-1} was observed. TYL on the other hand showed a K_d value ranging from 5.1 ± 2.29. TYL sorption on soil has been studied and an equilibrium K_d of 8.3 L kg^{-1} was observed (Rabolle et al., 2000). The K_d for SMZ differs from the results from Ilhan et al., (2010) by ten times. The possible reasons could be that the woodchips they used were collected from a denitrification wall in 2004 at a depth of 170 cm and thus could have differed in the available organic carbon content for sorption. The major reason for such a huge difference in the K_d value is the solid to liquid ratio. They used one gram woodchip in a test tube with a concentration of 0.83 mg L^{-1} of SMZ which is very less as compared to our solid-liquid ratio. It was noted however that the slope or the K_d for TYL in Figures 4.6 and 4.7 of the 24 hour sample is smaller than that of the 12 hour test and the 12 hour slope is smaller than the 6 hour sample for SMZ in Figures 4.4 and 4.5. Desorption data showed desorption was occurring because there is an increase in the concentration of SMZ in the aqueous phase after 24 hours of fresh water being added showing that the antibiotic is released in the aqueous phase from the solid phase; however a clear trend was only developed with SMZ after 7 days (Figure 4.7). The AHI for this data calculated at a liquid concentration of 1.0 mg L^{-1} was 0.0336 which meant very little to no hysteresis occurred implying the sorption process was reversible. Reversible sorption is shown by the similarity in aqueous phase concentration after sorption and desorption and absence of hysteresis represents the original state of the liquid phase concentration after desorption.

Fig 4.8 (a)

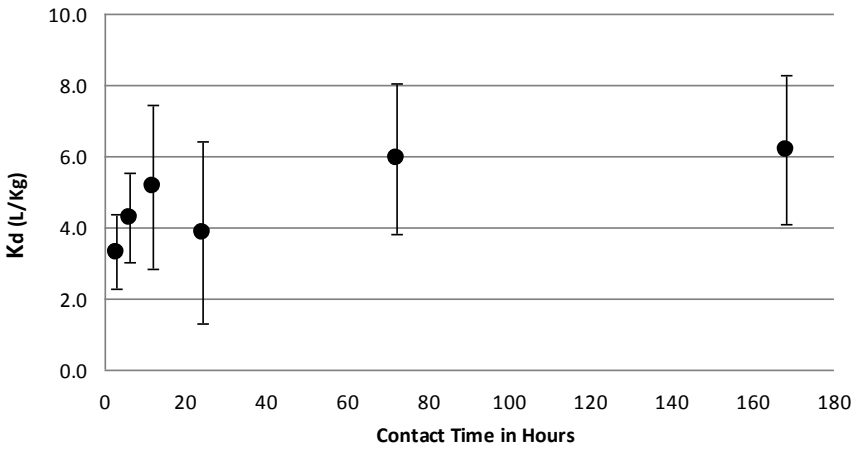

Fig 4.8 (b)

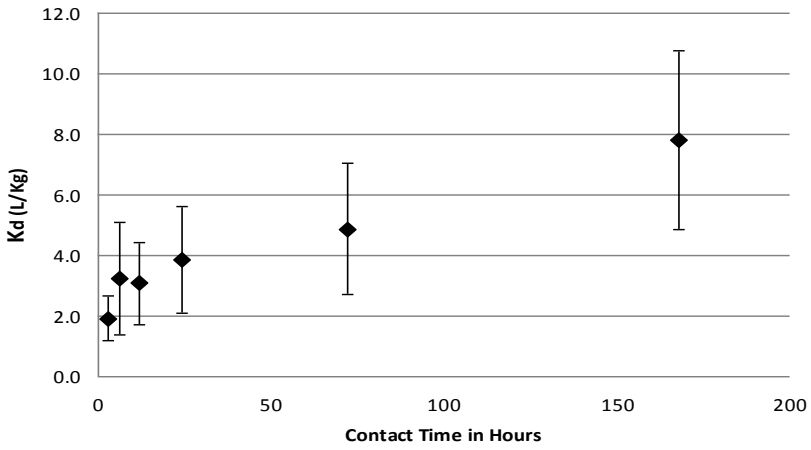

Figure 4-8: Time Dependent Changes in K_d in Old Woodchips for a) Tylosin, and b) Sulfamethazine.

Note: Error bars represent the standard deviation of a data point from mean, calculated from triplicates. It denotes the interval in which the mean of data lies.

The comparison of sorption between new and old woodchips was not possible because the data for the new woodchips were inconclusive. The variation and extra peaks in the chromatographs during the HPLC analysis was due to the dissolved contaminants that remained in the liquid phase after the pre wash of woodchips. Regression analysis was performed using SAS (Statistical Analysis Software) and it was concluded that both sorption and desorption isotherms have same slopes. Solid phase concentration was regressed on aqueous phase concentration. Confidence intervals for the regression coefficients were found to be overlapping. For sorption the 95% confidence interval for the regression coefficient was (3.8, 13.6) and that for desorption was (7.1, 9.4). Therefore, it was concluded that the slopes of the two isotherms were not different which also confirms reversible sorption.

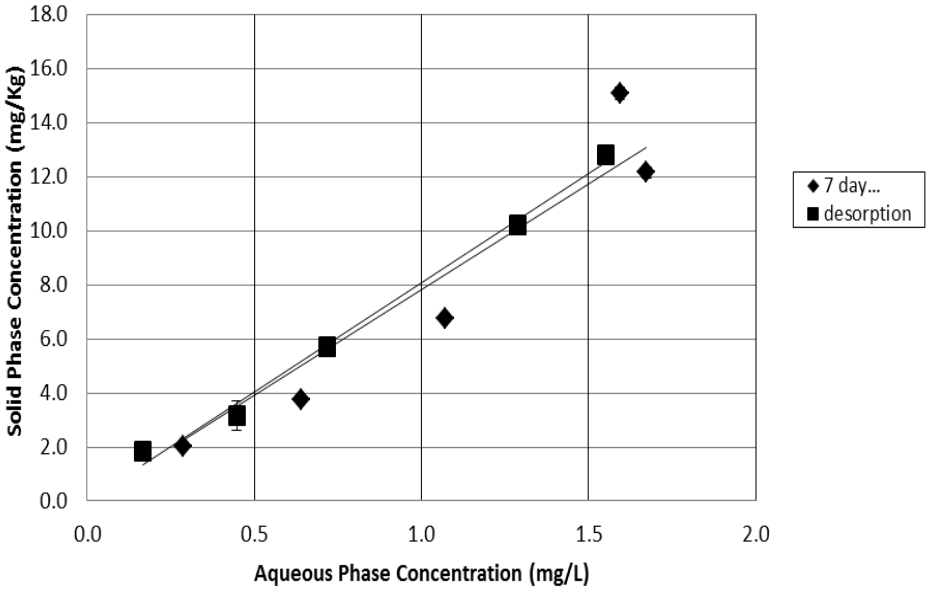

Figure 4-9: Sorption and Desorption Isotherms for 7 day Contact Time for Sulfamethazine.

Note: Error bars represent the standard deviation of a data point from mean, calculated from triplicates. It denotes the interval in which the mean of data lies.

The increase of slope or K_d values on the phase distribution relationship (PDR) graphs indicates increased sorption with an increase in contact time. The discrepancies of the 24 hour data points for TYL and the 12 hour points for SMZ could be due to experimental variability. The desorption experiment shows a reversible sorption for SMZ. Thus it can be predicted that a woodchip bioreactor would now be efficient in removing SMZ from water sources, It may slow the transport of SMZ into the streams and consequently into the rivers but would eventually desorb in the water.

The typical retention times for bioreactors are in the range of 4 to 8 hours which is much lower than the equilibrium contact time of 7 days noted in this study thus the percent loss of TYL and SMZ concentration was calculated for a retention time of 3 hours for an initial concentration of 10 mg L^{-1} and a loss of 75% and 76% for SMZ and TYL on old woodchips and approximately 40% and 30% for SMZ and TYL concentration on fresh woodchips was found showing a substantial sorption even in 3 hours which shows that sorption process would be effective in the typical range of retention times in a bioreactor. Using these K_d values, amount of woodchips needed in the installation of a bioreactor can be determined. Flow rate and influent concentration of antibiotic can be measured and using the flow rate the retention time (τ) of the bioreactor can be calculated as:

$$\tau = \frac{\rho V}{Q}$$

Where V = bioreactor volume; ρ = porosity of the woodchips; and Q = volumetric flow rate through the bioreactor. From lab experiments, using standard concentrations, the effluent concentration and solid phase concentration can be determined by developing sorption isotherms and predicting for in field concentrations. Using all the above data and the K_d for equilibrium,

approximate amount of woodchip required at equilibrium can be predicted by substituting all the known values in calculations for K_d, only mass of woodchip would be unknown.

The possible occurrence of desorption of antibiotics in the bioreactor may cause the antibiotics to reenter the tile water or groundwater. The presence of desorption could be easily observed in the experiment but only SMZ showed an anticipated linear trend to the left of the sorption rate after 7 days (Figure 4.7). The desorption data for TYL could not be determined because of inconsistency in the analysis of the HPLC. An AHI value of 0.0336 was calculated which meant very little to no hysteresis occurred which implied that solid phase concentrations obtained from sorption and desorption did not differ very much. As mentioned earlier, a value of zero indicates no hysteresis is occurring (Bhandari and Lesan, 2003). Thus, the calculated index indicates presence of reversible sorption.

Batch experiments for sorption of TYL and SMZ were also conducted at the Environmental Engineering Research Lab at Kansas State University. PDRs could not developed for TYL and SMZ for both old as well as fresh woodchips due to lack of data. One reason was the minimum detection limit of the HPLC was approximately 1 mg L^{-1} because of which the data for the sorption of 1, 3 and 5 mg L^{-1} could not be determined properly even after a sorption period of 3 hours. Data for 8 and 10 mg L^{-1} was fairly consistent but a K_d could not be developed using the two data points.

4.2 Effect of Woodchips Particle Size on Sorption

The percent loss for TYL and SMZ conducted at Water Quality Research Lab, Iowa State University, for each old woodchip particle size is shown in Figure 4.10. The woodchips were

separated into 3 different sizes by manually cutting them to an approximate average size of 4.0 cm x 1.0 cm and fine particle sizes of approximately 0.5 cm x 0.5 cm. The percent loss for each woodchip size and both chemicals was calculated. After 7 days of experiment, the loss in all the cases reached around 90% which gives us unexpected results as we expect the sorption to increase as the size decreases i.e. a decrease in size means an increase in the surface area hence more 'open' sites for sorption. In our results, the sorption for all the sizes is nearly the same which makes us believe that sorption is not changed much by the size of wood. Thus it shows more absorption than adsorption. If sorption was being varied by the surface of woodchips we should have seen more sorption in smaller woodchips but there seems to be same sorption in all the three cases which implies more absorption. The difference in size of the woodchips was not significantly large which implies that there was not a significant increase in the total sorbent surface area to have high impact of sorption. Thus more research is required to confirm the variation sorption on woodchip of much smaller size.

The percent loss for TYL and SMZ conducted at Environmental Engineering Research lab, Kansas State University for each old woodchip particle size is shown in Figure 4.11 and for fresh woodchips in Figure 4.12. The woodchips were separated into 3 different sizes by manually cutting them to an approximate average size of 4.0 cm x 1.0 cm and fine particle sizes of approximately 0.5 cm x 0.5 cm. The volume of the serum bottles used were 160 mL and thus the solid to liquid ratio was adjusted to 27 gm in 94 mL. The percent loss for each woodchip size and both chemicals was calculated and after 10 days of experiment the loss in all the cases for old woodchips reached around 99% which reinforced the previous results showing that there is not much difference in sorption in the 3 cases. Furthermore, the percent loss for each woodchip size and both chemicals was calculated and after 10 days of experiment the loss in all the cases for

fresh woodchips reached around 90% which was as expected lesser sorption than the old woodchips but almost the same loss of SMZ and TYL concentration in all the 3 cases. Hence, it can be safely concluded that size of woodchip we used did not vary the amount of sorption to even a small extent and absorption plays the major role in the sorption of the antibiotics than adsorption. It was also observed that the difference in size of the woodchips was not significantly large which implies that there was not a significant increase in the total sorbent surface area to have high impact of sorption. Approximately 40-50 woodchips of the size 4.0 cm x 1.0 cm were fit in the bottle as compared to approximately 100-120 woodchips of the size 2.5 cm x 1.0 cm and 350-450 of the size 0.5 cm x 0.5 cm. When the woodchips were manually cut, the increase in the surface area was along the height (approximately 0.2 cm). The increase in surface area along the height was approximately twice for the woodchip size 2.5 cm x 1.0 cm and 6-8 times for the size 0.5 cm x 0.5 cm. No effect on TYL and SMZ was observed even when the wood was cut into 2.5 cm x 1.0 cm (which corresponds to an inc. of 10% - 15 %. For 0.5 x 0.5 cm wood, the length and width are so small that it does not affect the surface area to higher extent as we are limited by the number of small woodchips that can be added to the bottle.

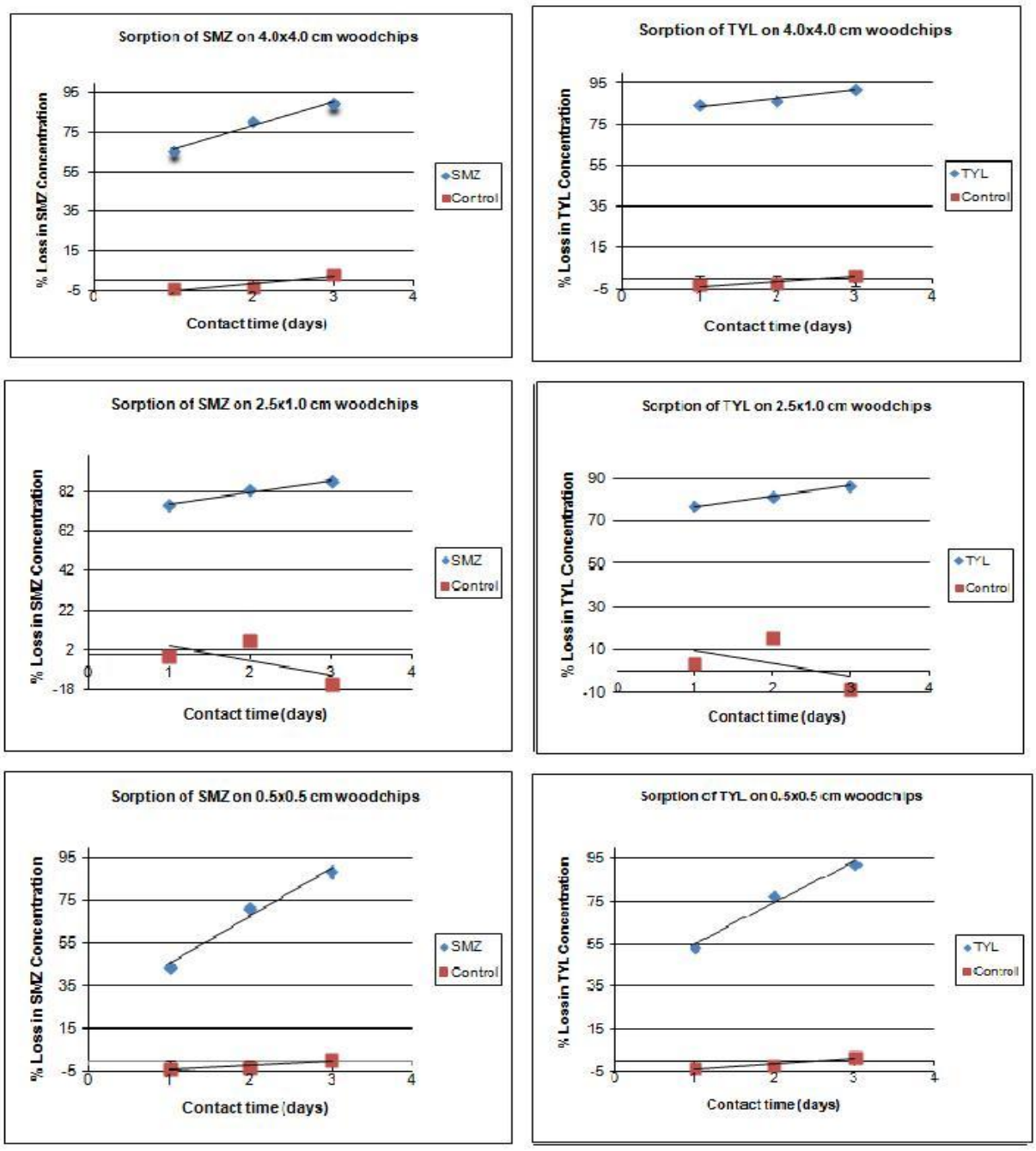

Figure 4-10: Percent Loss of Tylosin and Sulfamethazine on Different Size Woodchips (Old Woodchips) Solid to Liquid Ratio: 20 gm in 70 mL

Note: Error bars represent the standard deviation of a data point from mean, calculated from triplicates. It denotes the interval in which the mean of data lies.

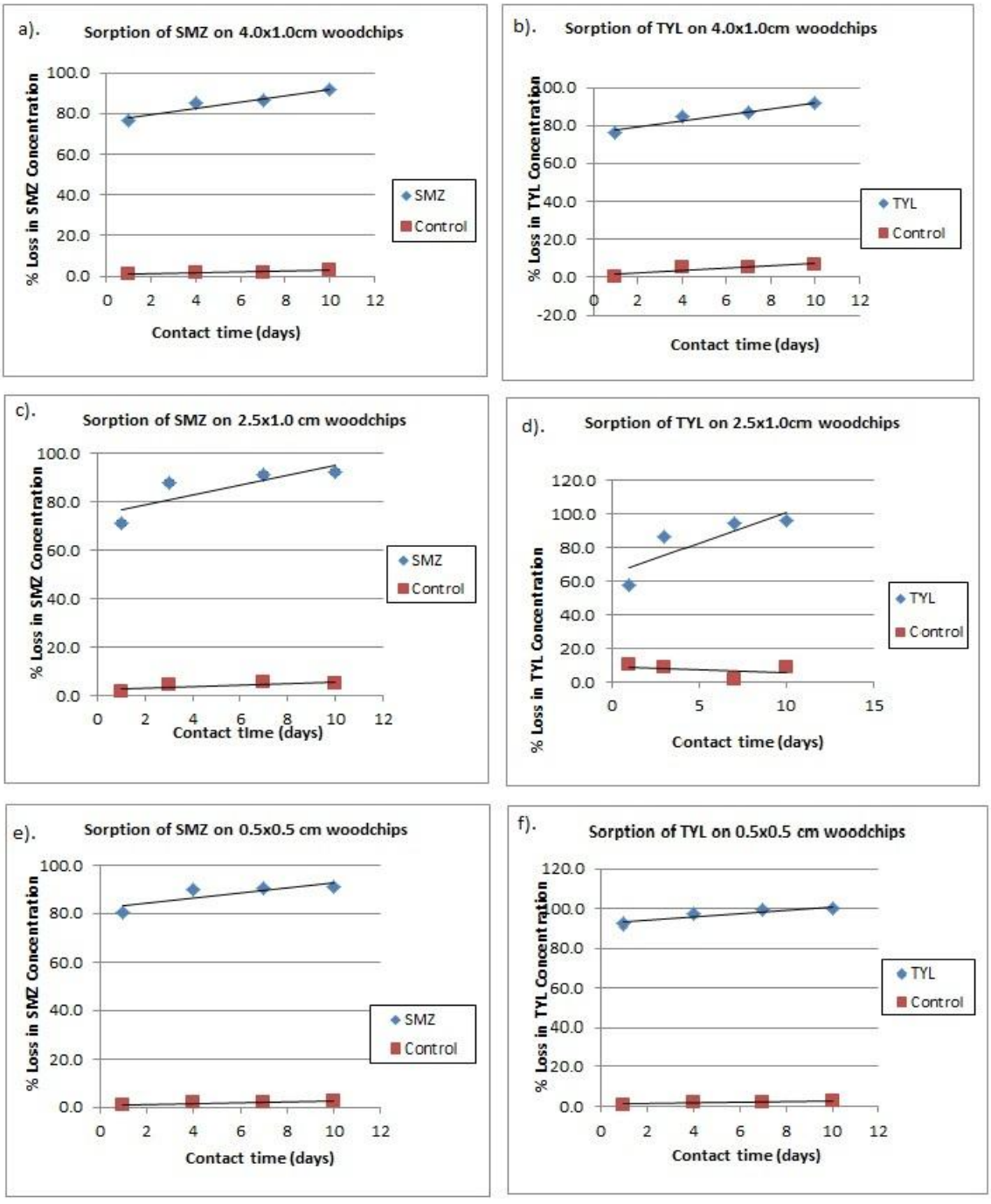

Figure 4-11: Percent Loss of Tylosin and Sulfamethazine on Different Size Woodchips (Old Woodchips) Solid to Liquid Ratio: 27 gm in 94 mL.

Note: Error bars represent the standard deviation of a data point from mean, calculated from triplicates. It denotes the interval in which the mean of data lies.

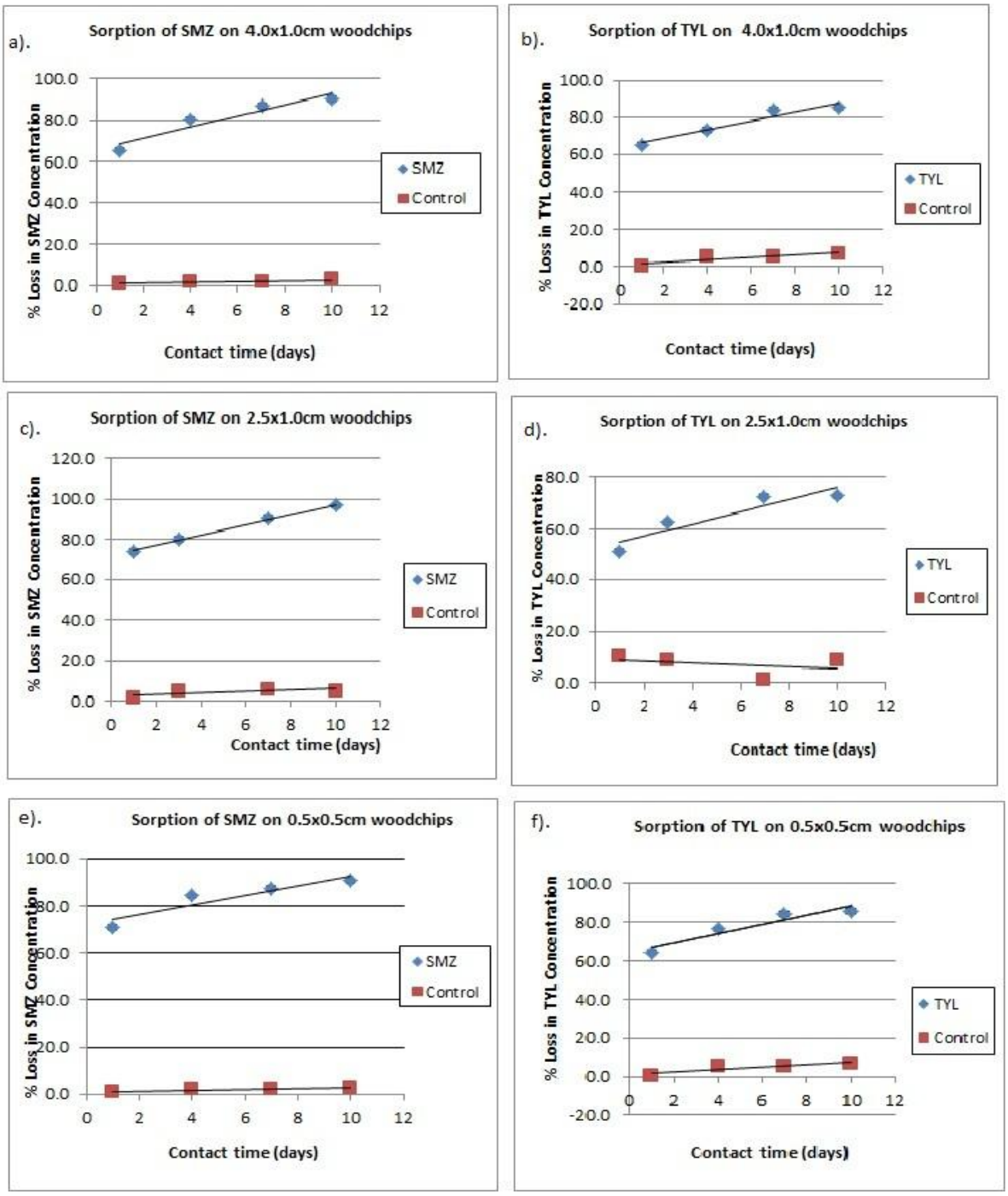

Figure 4-102: Percent Loss of Tylosin and Sulfamethazine on Different Size Woodchips (Fresh Woodchips), Solid to Liquid Ratio: 27 gm in 94 mL.

Note: Error bars represent the standard deviation of a data point from mean, calculated from triplicates. It denotes the interval in which the mean of data lies.

Chapter 5 - Conclusion

The veterinary antibiotics that enter the environment via direct application of manure may impact the water and environmental quality. The major concern is the contamination of groundwater with veterinary antibiotics and growth of resistant micro-organisms which can potentially have severe health effects. The woodchip bioreactors which are used to remove nitrates from tile drainage water may also reduce the transport of pharmaceuticals. In this work, sorption-desorption of Tylosin (TYL) and Sulfamethazine (SMZ) to woodchips was investigated. Both of the antibiotics were observed to have a high loss of concentration in the 7 day contact time during sorption in woodchips. This shows that woodchip bioreactors may be an effective way to remove the antibiotics from the tile drainage. Experiments were also performed to see the variation in sorption due to change in size and it was concluded that the three size of woodchips did not differ the sorption. Our observation was that the difference in size of the woodchips was not significantly large which implies that there was not a significant increase in the total sorbent surface area to have high impact on sorption. No affect on TYL and SMZ was observed even when the wood was cut into 2.5 cm x 1.0 cm (which corresponds to an increase of 10% - 15 %. For 0.5 x 0.5 cm wood, the length and width are so small that it does not affect the surface area. Results of desorption experiments of the two selected chemicals confirmed the presence of desorption in the woodchips. It was observed that desorption occurred with very low AHI, which logically predicts that the woodchips have reversible sorption of antibiotics. It was found that the woodchips do have a potential to sorb the antibiotics but desorption of antibiotics also takes place. Thus, it shows that antibiotics entering the bioreactor through tile drainage may eventually desorb from the wood and end up in the streams. This concludes that a woodchip

bioreactor may not be an effective way to remove the antibiotics from the tile drainage. For both sulfamethazine and tylosin in old woodchips, the linear partition coefficient was >7.8 L kg^{-1} and 5.1 ± 2.29 L kg^{-1} respectively. Finally, the experiments performed in this study were at room temperature, whereas the temperature of tile water is much lower. Expected temperature in field is about 10 °C which is lower than room temperature. The lower temperature might decrease $K_{d.}$ Thus, variation in sorption and desorption on woodchips at much lower temperature of tile water should also be studied.

Appendix A - Data for Loss of Tylosin and Sulfamethazine due to Sorption on Fresh Woodchips (Solid to Liquid ratio: 25 gm / 70 ml.)

Contact time	SMZ Loss %	TYL Loss%
1day	30.9	36.4
3 days	49.3	42.2
7 days	56.1	44.3

Appendix B - Data for Loss of Tylosin and Sulfamethazine due to Sorption on Old Woodchips (Solid to Liquid ratio: 25 gm / 70 ml.)

Contact time	SMZ Loss %	TYL Loss%
1day	89.7	83.4
3 days	97.6	83.5
7 days	97.3	81.0

Appendix C - Phase Distribution Relationships of Sulfamethazine on Old Woodchips for 3, 6 and 12 Hour Contact Times (Solid to Liquid Ratio: 25 gm in 70 mL)

3 Hour Sulfamethazine Sorption	Aqueous Concentration	Solid Phase Concentration
1	0.8	2.8
3	1.1	1.2
5	2.0	2.8
8	2.3	5.7
10	3.5	6.5
6 Hour Sulfamethazine Sorption	**Aqueous Concentration**	**Solid Phase Concentration**
1	0.5	0.5
3	0.8	2.3
5	0.8	3.9
8	1.8	6.0
10	2.4	7.4
12 Hour Sulfamethazine Sorption	**Aqueous Concentration**	**Solid Phase Concentration**
1	0.3	2.1
3	1.8	1.7
5	0.9	4.4
8	1.9	6.9
10	2.4	8.7

Appendix D - Phase Distribution Relationships of Sulfamethazine on Old Woodchips for 24, 72 and 168 Hour Contact Times (Solid to Liquid Ratio: 25 gm in 70 mL)

24 Hour Sulfamethazine Sorption	**Aqueous Concentration**	**Solid Phase Concentration**
1.0	0.9	3.3
3.0	0.7	3.2
5.0	1.7	4.8
8.0	2.4	9.3
10.0	2.7	11.4
72 Hour Sulfamethazine Sorption	**Aqueous Concentration**	**Solid Phase Concentration**
1.0	0.4	3.3
3.0	0.6	2.6
5.0	1.6	4.8
8.0	2.1	10.7
10.0	2.0	11.8
168 Hour Sulfamethazine Sorption	**Aqueous Concentration**	**Solid Phase Concentration**
1.0	0.3	2.0
3.0	0.6	3.8
5.0	1.1	6.7
8.0	1.7	12.2
10.0	1.6	15.1

Appendix E - Phase Distribution Relationships of Tylosin on Old Woodchips for 3, 6 and 12 Hour Contact Times (Solid to Liquid Ratio: 25 gm in 70 mL)

3 Hour Tylosin Sorption	Aqueous Concentration	Solid Phase Concentration
1	0.7	0.7
3	0.9	2.3
5	1.4	4.7
8	1.1	8.5
10	3.3	9.9
6 Hour Tylosin Sorption	**Aqueous Concentration**	**Solid Phase Concentration**
1	0.7	0.7
3	0.9	3.3
5	0.9	5.1
8	1.3	8.4
10	2.5	9.7
12 Hour Tylosin Sorption	**Aqueous Concentration**	**Solid Phase Concentration**
1	0.7	0.7
3	0.8	3.2
5	0.9	5.4
8	1.7	8.8
10	2.0	11.3

Appendix F - Phase Distribution Relationships of Tylosin on Old Woodchips for 24, 72 and 168 Hour Contact Times (Solid to Liquid Ratio: 25 gm in 70 mL)

24 Hour Tylosin Sorption	**Aqueous Concentration**	**Solid Phase Concentration**
1.0	1.3	0.4
3.0	1.2	3.2
5.0	1.7	5.7
8.0	2.3	11.2
10.0	3.0	13.0
72 Hour Tylosin Sorption	**Aqueous Concentration**	**Solid Phase Concentration**
1.0	0.6	1.3
3.0	1.0	3.5
5.0	1.2	6.6
8.0	1.5	11.6
10.0	2.0	12.4
168 Hour Tylosin Sorption	**Aqueous Conc.**	**Solid Phase**
1.0	0.7	0.7
3.0	0.9	4.0
5.0	1.5	6.9
8.0	2.2	12.4
10.0	1.8	16.8

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Appendix G - Time Dependent Changes in K_d in Old Woodchips for Tylosin.

Time Dependent Changes in K_d in Old Woodchips for Tylosin	
Time	**Kd**
3	1.9
6	3.2
12	3.1
24	3.8
72	4.9
168	7.8

Appendix H - Time Dependent Changes in K_d in Old Woodchips for Sulfamethazine.

Time Dependent Changes in K_d in Old Woodchips for Sulfamethazine	
Time	**K_d**
3	3.3
6	4.3
12	5.1
24	3.9
72	5.9
168	6.2

Appendix I - Sorption and Desorption Isotherms for 7 day Contact Time for Sulfamethazine

Sorption and Desorption Isotherms for 7 day Contact Time for Sulfamethazine				
Contact Time	Sorption		Desorption	
	c(t)	q(t)	c(t)	q(t)
1	0.3	2.0	0.2	1.8
3	0.6	3.8	0.4	3.2
5	1.1	6.7	0.7	5.7
8	1.7	12.2	1.3	10.2
10	1.6	15.1	1.5	12.8

Appendix J - Percent Loss of Tylosin and Sulfamethazine on Different Size Woodchips (Old Woodchips) Solid to Liquid Ratio: 20 gm in 70 mL

	4cmx1cm		2.5cmx1cm		0.5cmx0.5cm	
Contact Time	SMZ Loss %	TYL Loss %	SMZ Loss %	SMZ Loss %	TYL Loss %	SMZ Loss %
Day 1	65.9	84.03	74.8	76.1	43.7	53.4
Day 2	80.7	85.7	83.1	80.3	71.5	77.2
Day 3	89.7	92.1	87.6	87.8	88.1	92.7

Appendix K - Percent Loss of Tylosin and Sulfamethazine on Different Size Woodchips (Old Woodchips) Solid to Liquid Ratio: 27 gm in 94 mL.

	4cmx1cm		2.5cmx1cm		0.5cmx0.5cm	
Contact Time	SMZ Loss %	TYL Loss %	SMZ Loss %	TYL Loss %	SMZ Loss %	TYL Loss %
Day 1	78.4	78.4	72.4	59.1	80.2	91.1
Day 2	82.3	82.6	87.1	84.5	88.1	97.6
Day 3	83.1	83.1	88.3	97.1	88.5	98.2
Day 10	90.6	90.1	89.1	98.2	88.6	99.6

Appendix L - Percent Loss of Tylosin and Sulfamethazine on Different Size Woodchips (Fresh Woodchips), Solid to Liquid Ratio: 27 gm in 94 mL.

	4cmx1cm		2.5cmx1cm		0.5cmx0.5cm	
Contact Time	SMZ Loss %	TYL Loss %	SMZ Loss %	Contact Time	SMZ Loss %	TYL Loss %
Day 1	63.4	63.1	78.4	50.6	70.2	62.6
Day 2	80.4	70.5	80.4	61.4	82.5	78.5
Day 3	83.6	82.3	90.7	70.5	83.1	81.2
Day 10	90.1	82.7	98.3	70.6	84.6	81.9

Appendix M - SAS Output (Regression of Kd of Tylosin on time) and t-test on dataset for Kd of Tylosin

Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	4.17874	4.17874	6.99	0.0573
Error	4	2.38960	0.59740		
Corrected Total	5	6.56833			

Root MSE	0.77292	R-Square	0.6362
Dependent Mean	4.78333	Adj R-Sq	0.5452
Coeff Var	16.15852		

Parameter Estimates					
Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	4.10739	0.40606	10.12	0.0005
time	1	**0.01423**	0.00538	2.64	0.**0573**

t-test on dataset for K_d of Tylosin:

P value and statistical significance:

The two-tailed P value equals 0.1771

By conventional criteria, this difference is considered to be not statistically significant.

Confidence interval:

The mean of Group One minus Group Two equals -0.80360

95% confidence interval of this difference: From -2.16726 to 0.56006

Intermediate values used in calculations:

t = 1.6361

df = 4

standard error of difference = 0.491

Review your data:

Group	Group One	Group Two
Mean	5.05800	5.86160
SD	2.26098	2.13230
SEM	1.01114	0.95360
N	5	5

Appendix N - SAS Output (Regression of Kd of Sulfamethazine on time) and t-test on dataset for K_d of Sulfamethazine

Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	20.05140	20.05140	78.87	0.0009
Error	4	1.01694	0.25423		
Corrected Total	5	21.06833			

Root MSE	0.50422	R-Square	0.9517
Dependent Mean	4.11667	Adj R-Sq	0.9397
Coeff Var	12.24818		

Parameter Estimates					
Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	2.63600	0.26490	9.95	0.0006
time	1	**0.03117**	0.00351	8.88	0.**0009**

t-test on dataset for K_d of Sulfamethazine:

P value and statistical significance:

The two-tailed P value equals 0.0029

By conventional criteria, this difference is considered to be very statistically significant.

Confidence interval:

The mean of Group One minus Group Two equals -3.098540

95% confidence interval of this difference: From -4.425781 to -1.771299

Intermediate values used in calculations:

t = 6.4818

df = 4

standard error of difference = 0.478

Review your data:

Group	Group One	Group Two
Mean	4.738800	7.837340
SD	2.138978	2.942556
SEM	0.956580	1.315951
N	5	5

Appendix O - SAS Output (Sorption)

Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	112.60955	112.60955	31.39	0.0112
Error	3	10.76245	3.58748		
Corrected Total	4	123.37200			

Root MSE	1.89407	R-Square	0.9128
Dependent Mean	7.96000	Adj R-Sq	0.8837
Coeff Var	23.79479		

Parameter Estimates					
Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	-1.24893	1.84910	-0.68	0.5478
c1	1	8.68767	1.55064	5.60	0.0112

Following formula was used to compute confidence interval for the regression coefficient:

Confidence interval for a regression coefficient:

$$\beta_j \pm t_{(\frac{1-\alpha}{2},n-k-1)} SE_{\beta_j}$$

where β_j is the value of the regression coefficient for independent variable j, α is the desired confidence interval percentage, $SE_{\beta j}$ is the standard error for β_j, t is a t-value, k is the number of predictors in the model, and n is the total sample size.

A 95% confidence interval for the regression coefficient is: $3.75284 \leq \beta \leq 13.62250$

Appendix P - SAS Output (Desorption)

Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	86.22077	86.22077	526.56	0.0002
Error	3	0.49123	0.16374		
Corrected Total	4	86.71200			

Root MSE	0.40465	R-Square	0.9943
Dependent Mean	6.74000	Adj R-Sq	0.9924
Coeff Var	6.00374		

Parameter Estimates					
Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	-0.02177	0.34580	-0.06	0.9538
c2	1	8.24606	0.35935	22.95	0.0002

Following formula was used to compute confidence interval for the regression coefficient:

Confidence interval for a regression coefficient:

$$\beta_j \pm t_{(\frac{1-\alpha}{2},n-k-1)} SE_{\beta_j}$$

where β_j is the value of the regression coefficient for independent variable j, α is the desired confidence interval percentage, $SE_{\beta j}$ is the standard error for β_j, t is a t-value, k is the number of predictors in the model, and n is the total sample size.

A 95% confidence interval for the regression coefficient is: $7.10245 \leq \beta \leq 9.38967$