Fate and Impact of Antibiotics in Slow-rate Biofiltration Processes

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For redemption and purpose I am sustained by Jesus Christ, the creator of the universe who gives abundantly.

"He was supreme in the beginning and—leading the resurrection parade—he is supreme in the end. From beginning to end he's there, towering far above everything, everyone. So spacious is he, so roomy, that everything of God finds its proper place in him without crowding. Not only that, but all the broken and dislocated pieces of the universe—people and things, animals and atoms—get properly fixed and fit together in vibrant harmonies, all because of his death, his blood that poured down from the cross."

–Colossians 1: 18-20

The Message (E. Peterson, 2002, Intervarsity Press, Downers Grove, IL, USA)

ABSTRACT

Antibiotics have been detected in surface waters worldwide at concentrations up to 1.9 μg/L, but are typically detected at low ng/L concentrations. The potential health effects of exposure to low levels of these compounds via tap water are not known, but there is significant concern among water consumers regarding the occurrence of antibiotics and other pharmaceutical compounds in water supplies. Thus, a significant amount of research has been performed recently to investigate the removal of pharmaceuticals via conventional and advanced water treatment processes. While conventional treatment processes (*i.e.*, coagulation, flocculation, sedimentation, and filtration) are generally not effective, oxidation processes (*e.g.*, chlorination, ozonation) and granular activated carbon exhibit some effectiveness at removing pharmaceuticals. As expected, removals are highly dependent on compound structure. Furthermore, some oxidants, such as chloramines, are not effective at oxidizing pharmaceuticals.

Slow-rate biofiltration processes (SRBF), such as slow sand filtration (SSF) and riverbank filtration (RBF), are drinking water treatmeant systems comprised of two stages in sequence: 1) a relatively shallow biotic region where media (*i.e.*, filter sand or aquifer material) is colonized by biofilm bacteria, followed by 2) an deeper abiotic filtration zone. These processes are extensively used in Europe and developing global regions and are seeing increased usage in the United States. There is evidence in the literature that SRBFs can remove a wide variety of trace organic pollutants including: pesticides, disinfection byproducts, and some pharmaceuticals. Little is known regarding

the ability of SRBF processes to remove antibiotics from water supplies nor has any work been done to investigate the potential adverse effects of antibiotics on the biofilm bacteria that are critical to SRBF system performance. Thus, this research was performed to determine the extent and mechanisms (*i.e.*, sorption versus biodegradation) of antibiotic removal in SRBF processes and the effects of antibiotics on biofilm bacteria (*i.e.*, activity and community composition).

The effect of antibiotics on bacterial activity and community structure was investigated by growing biofilm in the presence and absence of a mixture of antibiotics in a continuous-flow rotating annular bioreactor (CFRAB) with acetate as substrate. Three representative compounds were selected for use in this research: sulfamethoxazole (SMX), erythromycin (ERY), and ciprofloxacin (CIP). These antibiotics were selected because they: 1) represent three prominent classes of antibiotics with differing mechanisms of action against bacteria, 2) have been detected in surface water, 3) exhibit different chemical characteristics, and 4) have differing levels of biodegradability. Areal acetate utilization rates for a constant feed of antibiotics were similar to the control experiments, and utilization rates did not change during an antibiotic shock loading experiment. Attached biomass levels were greater for experiments involving a "high CIP" concentration (3.33 μg/L), however, yielding comparatively lower steady-state biomass-normalized substrate utilization rates. Microbial community analyses via automated ribosomal intergenic spacer analysis (ARISA) revealed shifts in community structure for the high dose CIP experiments.

A CFRAB was also used to investigate antibiotic sorption to bacterial biofilm. The extent of sorption, as indicated by the organic carbon partition coefficient (K_{oc}), was 15 to 23 times greater for CIP compared to ERY and SMX. The K_{oc} values did not correlate with experimentally-determined K_{ow} values, suggesting that the sorption of relatively hydrophilic (*i.e.* $K_{ow} < 1.7$) and charged antibiotics to typically negatively charged biofilm is driven by ionic interactions (*i.e.* ion exchange) rather than hydrophobic interactions.

The attenuation and impact of antibiotics in SRBF systems was investigated by conducting bench-scale filter column experiments with mixtures of SMX, ERY, and CIP at high (3.33 μg/L, each) and low (0.33 μg/L, each) antibiotic feed conditions. Consistent with the CFRAB experiments, antibiotic breakthrough times were greatest for CIP, with very little uptake of SMX or ERY. Biodegradation was not observed for any antibiotic during 6-weeks of filter column operation or in complementary batch experiments. A one-dimensional advection-dispersion equation (with linear sorption) model was validated against experimental results and used to compare antibiotic retardation in SSF, RBF, and rapid gravity biofiltration (RGBF) systems. Of the modeled systems, antibiotic retardation was greatest in RBF, with little antibiotic removal expected for SSF. Based on analysis of ARISA data, the community structure of bacterial biofilm was not affected in filters exposed to antibiotics at low concentrations (*i.e.* 0.33 μg/L, each) similar to those found in surface waters, with a few species impacted under high concentration conditions (3.33 μg/L, each).

The results of this work will help those interested in understanding and predicting antibiotic fate in engineered and natural systems where biofilm is present. The results indicate that antibiotic removal in SRBF processes will be dictated by compound properties such as charge and hydrophobicity, and that limited removal of antibiotics in SRBF processes can be expected. Finally, the results suggest that that mixtures of antibiotics at concentrations typically observed in surface waters are unlikely to adversely affect SRBF biofilm bacteria or process performance.

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1.1.1 Overview

Recent evidence indicates the widespread occurrence of antibiotics in surface waters worldwide. Antibiotics that are not metabolized during use enter the environment as excreted human or animal waste, with reported concentrations in the nanogram to microgram per liter range for surface water and nanogram to microgram per liter range for treated wastewater effluents (Hirsch *et al*., 1999a; Huang *et al.,* 2001*;* Kolpin *et al.*, 2002; Calamari *et al.*, 2003; Giger *et al.*, 2003; McArdell *et al.*, 2003; Miao *et al.*, 2004; Wiegel, 2004). Categorized according to their basic structure and mechanism of action, six antibiotic classes are commonly used in both human medicine and agricultural operations (*i.e*., growth promotion and prophylaxis), including aminoglycosides, β-lactams, macrolides, quinolones, sulfonamides, and tetracyclines (Huang *et al*., 2001). The environmental recalcitrance of antibiotics is partially due to the very properties that give them value in medicine. Designed for mobility in animal body tissue, antibiotics are usually hydrophilic (i.e., low logK_{ow}) and typically weak acids (sometimes polyprotic) that are often neutral or anionic at circumneutral pH (Table 1.1). Although antibiotic refers to compounds acting against bacteria that are naturally produced by microorganisms, herein, the term refers to both naturally produced and synthetic antibacterial compounds which is consistent with the common usage of the term.

The presence of antibiotics in surface waters is disconcerting for several reasons. The presence of low levels of antibiotics could lead to the development of antibiotic

Table 1.1. Major Antibiotic Classes (mechanisms of action) and representative compounds.

Macrolides (protein synthesis-50s subunit)

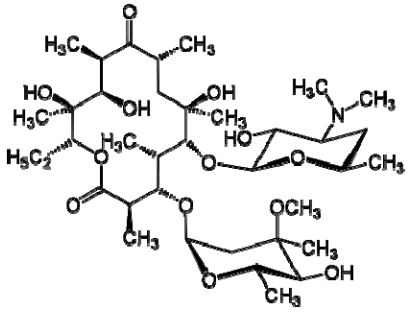
Erythromycin

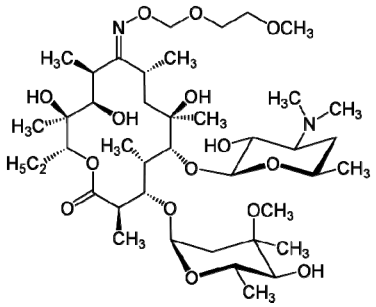
$C_{37}H_{67}NO_{13}$
$pK_A = 8.90$
MW = 733.9
$\log K_{ow} = 2.8$
$\log K_{ow}$ (pH 7.4) = 2.08

Roxithromycin

$C_{41}H_{76}N_2O_{15}$
$pK_A = 9.17$
MW = 837.1
$\log K_{ow} = 3.7$
$\log K_{ow}$ (pH 7.4) = 2.98

examples





Sulfonamides (folic acid production)

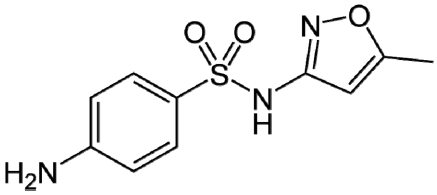
Sulfamethoxazole

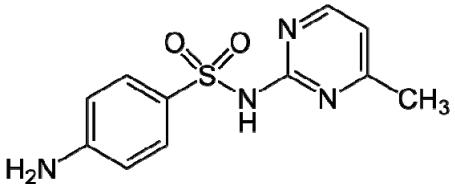
$C_{10}H_{11}N_3O_3S$
$pK_{A1} = 1.85$,
$pK_{A2} = 5.6$
MW = 253.3
$\log K_{ow} = 0.9$
$\log K_{ow}$ (pH 7.4) = -0.2

Sulfamerazine

$C_{11}H_{12}N_4O_2S$
$pK_{A1} = 2.06$,
$pK_{A2} = 6.90$
MW = 264.3
$\log K_{ow} = 0.3$
$\log K_{ow}$ (pH 7.4) = 0.0

examples





Quinolones (DNA gyrase production)

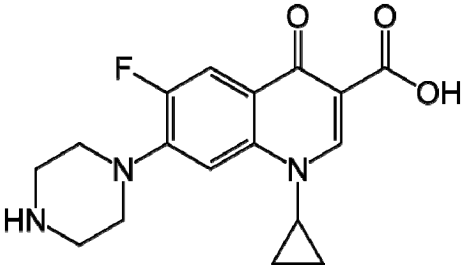
Ciprofloxacin

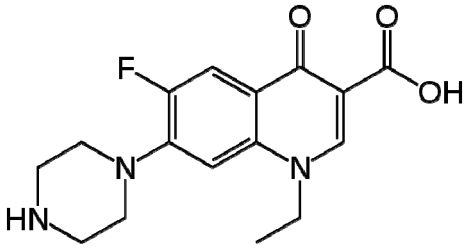
$C_{17}H_{18}FN_3O_3$
$pK_{A1} = 3.01$, $pK_{A2} = 6.14$
$pK_{A3} = 8.70$, $pK_{A4} = 10.58$
MW = 331.3
$\log K_{ow} = 0.7$
$\log K_{ow}$ (pH 7.4) = -1.35

Norfloxacin

$C_{16}H_{18}FN_3O_3$
$pK_{A1} = 3.11$, $pK_{A2} = 6.10$
$pK_{A3} = 8.60$, $pK_{A4} = 10.56$
MW = 319.3
$\log K_{ow} = 0.8$
$\log K_{ow}$ (pH 7.4) = -1.62

examples





Tetracyclines (protein synthesis-30s subunit)

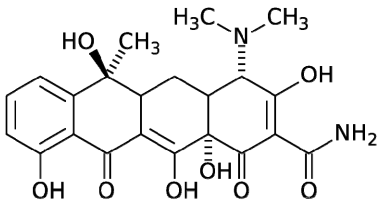
Tetracycline

$C_{22}H_{24}N_2O_8$
$pK_{A1} = 3.32$, $pK_{A2} = 7.78$
$pK_{A3} = 9.59$
MW = 444.4
$\log K_{ow} = -1.5$
$\log K_{ow}$ (pH 7.4) = -4.37

Oxytetracycline

$C_{22}H_{24}N_2O_9$
$pK_{A1} = 3.22$, $pK_{A2} = 7.48$
$pK_{A3} = 8.95$
MW = 460.4
$\log K_{ow} = -1.5$
$\log K_{ow}$ (pH 7.4) = -4.5

examples



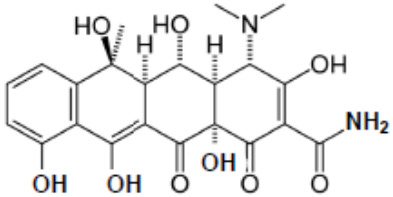


Table 1.1. (continued).

Aminoglycosides (Protein synthesis- 30S/50S subunit interface)

Streptomycin

$C_{21}H_{39}N_7O_{12}$
$pK_A = 7.8$[A]

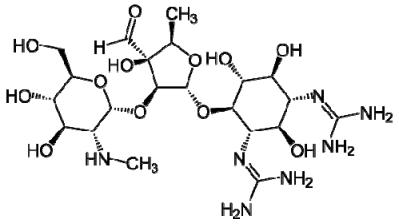
MW = 581.6
$\log K_{ow}$ = -2.7
$\log K_{ow}$ (pH 7.4) =-6.6

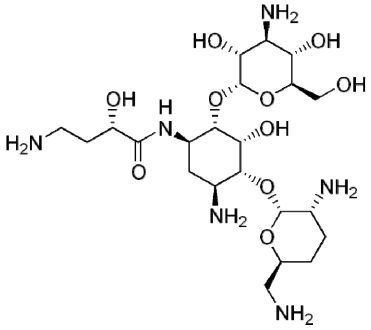
Arbekacin

$C_{22}H_{44}N_6O_{10}$
pK_A =8.1

MW = 552.62
$\log K_{ow}$ = -4.0
$\log K_{ow}$ (pH 7.4) = -10.4

examples





Glycopeptides (Peptidoglycan synthesis)

Vancomycin

$C_{66}H_{75}Cl_2N_9O_{24}$
$pK_A = 7.10$

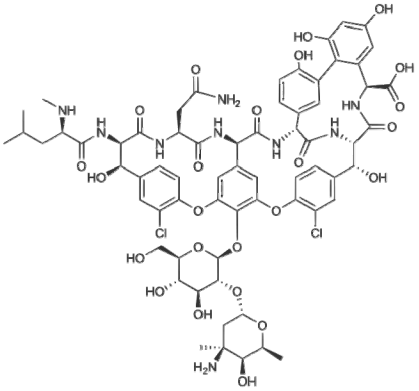
MW = 1449.3
$\log K_{ow}$ = -1.4
$\log K_{ow}$ (pH 7.4) = -4.52

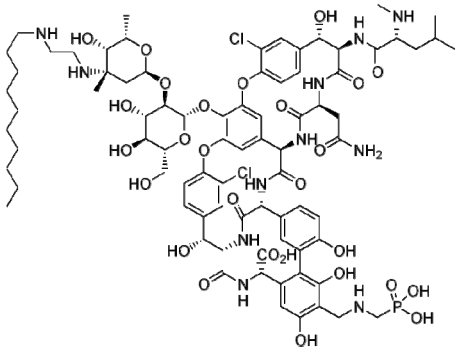
Telavancin[A]

$C_{80}H_{106}Cl_2N_{11}O_{27}P$
$pK_{A1} = 2.18$, $pK_{A2} = 7.75$
$pK_{A3} = 8.89$, $pK_{A4} = 9.54$
$pK_{A5} = 10.4$, $pK_{A6} = 12.0$

MW = 1755.63
$\log K_{ow}$ = 0.1
$\log K_{ow}$ (pH 7.4) = -2.37

examples





β-lactams/penicillin (cell wall synthesis)

Oxacillin

$C_{19}H_{19}N_3O_5S$
$pK_A = 2.72$[B]

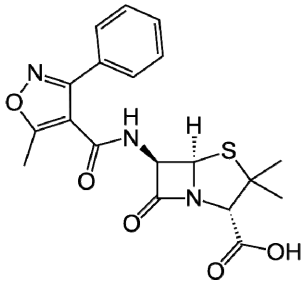
MW = 401.4
$\log K_{ow}$ = 2.1
$\log K_{ow}$ (pH 7.4) = -1.67

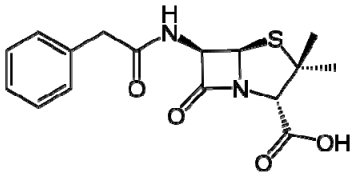
Penicillin G

$C_{16}H_{18}N_2O_4S$
pK_A =2.73[A]

MW = 334.4
$\log K_{ow}$ = 1.7
$\log K_{ow}$ (pH 7.4) = -2.1

examples





Note: pK_A values are from Qiang and Adams (2004) and $\log K_{ow}$ values are from O'Shea and Moser (2008), unless otherwise indicated.
[A]pK_A values from Takács-Novák *et al.* (1993)
[B]Prankerd (2007)

resistant bacteria (Armstrong *et al*., 1981; Ash *et al*., 2002; Schwartz *et al*., 2003). Also, mixtures of antibiotics at low concentrations (*i.e*., <50 μg/L) can adversely affect algae, nitrifying bacteria, and zooplankton (Flaherty and Dodson, 2005; Yang *et al*., 2008; Ghosh *et al*., 2009). And public awareness over the presence of antibiotics and other pharmaceutical compounds in drinking water supplies has been heightened (Benotti *et al*., 2009). Thus, there is significant interest in approaches to remove antibiotics and other pharmaceutical compounds from water supplies.

Conventional water treatment (*i.e*., coagulation/flocculation/sedimentation/filtration) or lime softening do not effectively remove all antibiotics (Adams *et al*., 2002; Westerhoff *et al*., 2005). Chemical oxidation processes (*e.g.*, chlorination, ozonation), however, can be effective at removing antibiotics from water. For example, carbodox, trimethoprim, and numerous sulfonamides in surface water exposed to free chlorine (1 mg/L for 40 minutes) were eliminated by more than 90%, but sulfamethoxazole (a sulfonamide) may reform during dechlorination (Adams *et al*., 2002; Dodd and Huang, 2004). Chlorine dioxide and ozone also effectively removed sulfonamides and macrolides (Huber *et al*., 2005; Westerhoff *et al*., 2005), but this was not the case for chloramines (Chamberlain and Adams, 2006). Granular activated carbon effectively removed sulfamethoxazole and erythromycin (Westerhoff *et al*., 2005; Snyder *et al*., 2007). Among membrane filtration alternatives only reverse osmosis and nanofiltration effectively reject antibiotics (Nghiem *et al*., 2005; Snyder *et al*., 2007). The fate of antibiotics in drinking water biofiltration systems has received little attention.

1.1.2 Slow-Rate Biofiltration Processes

1.1.2.1 Background

Slow-rate biofiltration (SRBF) processes such as slow sand filtration (SSF) and riverbank filtration (RBF) have been used worldwide for drinking water production. The earliest documented use of RBF dates to 1798 in Germany, with widespread application of SSF in Europe beginning in the latter half of the 19th Century (Rachwal *et al.,* 1996; Jekel and Grischek, 2003). While SRBF processes have been broadly and historically used in metropolitan areas of Europe (*e.g.*, RBF in Berlin, Amsterdam, Budapest, and SSF in London), more than 100 US utilities (*e.g.*, Hartford, CT, and Salem, OR) rely on SSF for water production, and there is increasing interest in RBF (*e.g.*, Cincinnati, OH, and Louisville, KY) as a first step for water treatment (Cleasby and Haarhof, 1991; Bouwer, 2003). There is also significant reliance on SSF water treatment in developing global regions (Hokanson *et al*., 2007).

1.1.2.2 Configuration and Composition

In SRBF systems, raw surface water is typically treated without prior processing (*i.e*., coagulation/flocculation/sedimentation); SRBF systems are the first barrier for removal of microbial pathogens and organic compounds. In SRBF systems, raw water is passed through engineered or natural porous media (*i.e*., filter sand or aquifer material) colonized by bacteria residing in biofilms. Typical filtration rates (and residence times) for SSF and RBF are 0.1 to 0.2 m/hr (2 to 4 days) and <0.1 m/hr (5 to 100 days), respectively (Kawamura, 1991; Kuehn and Mueller, 2000). Although these systems differ in temporal and spatial scale, they are similarly comprised of sequential biological and

abiotic filtration zones (Figure 1.1). The SSF biological component ("schmutzdecke") requires maturation periods ranging between 10 and 280 days prior to use, and is manually removed at prescribed intervals ranging between 1 to 3 months (Kawamura, 1991; Cleasby and Haarhof, 1991). The development of the RBF biological component ("colmation zone") is spatially dynamic and removed by natural hydraulic scour (Hiscock and Grischek 2002). The amount of biofilm and depth of the biological zone increases with time, depending on nutrient concentrations, suspended solids concentration, water temperature, and algal growth (Logsdon, 1991). The biological population is spatially dynamic and diverse, and comprised of diatoms, algae, protozoa, and some bacteria (Cleasby and Haarhof, 1991). Oxic conditions are typical through SSF systems, whereas RBF systems often progress from oxic to anoxic redox zones, especially for systems with longer residence times.

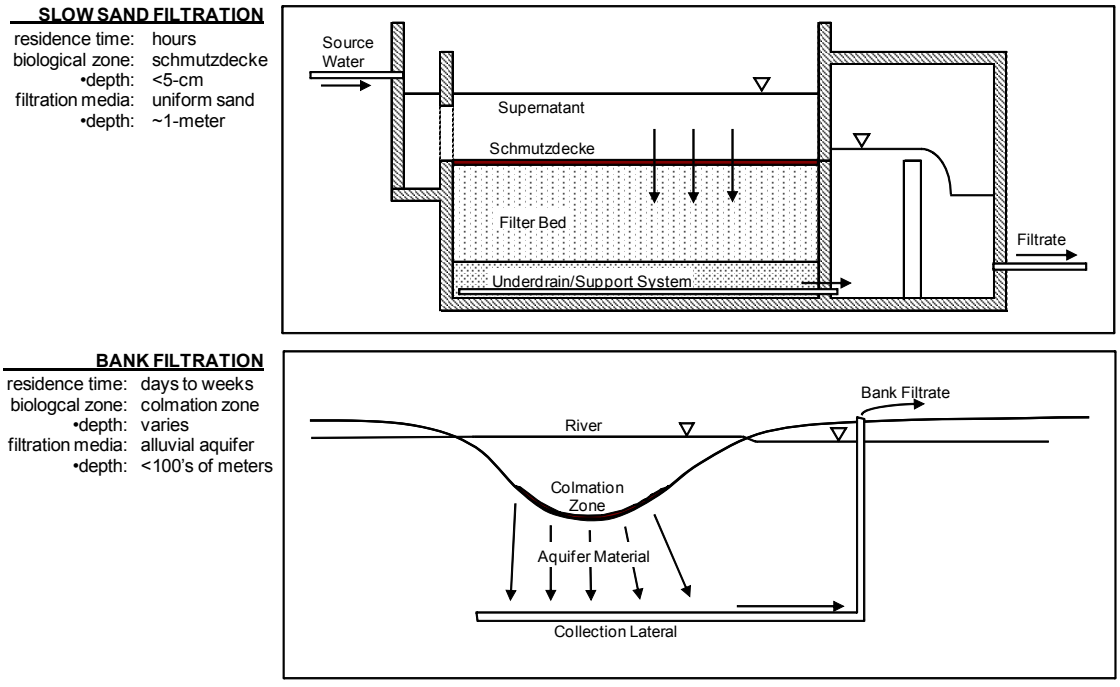


Figure 1.1. Comparison of Slow Sand and Bank Filtration SRBF Processes.

SRBF bacteria produce extracellular polymeric substances (EPS) that, in combination with humic substances (HS) derived from system water, constitute the biofilm that is attached to filtration media and provides structure and retention for bacteria. EPS is primarily comprised of proteins, carbohydrates, uronic acids, and DNA (Jahn and Nielsen 1995, Flemming *et al*., 1998). EPS is anionic and generally hydrophilic, but with hydrophobic groups (*e.g.*, methyl and acetyl) on the repeating polymer units (Bryers, 2000). EPS from heterotrophic bacteria exhibits acid pK_A values associated with carboxyl and phosphoric functional groups (Lee and Davis, 2001; Liu and Fang, 2002). HS are complex macromolecular residues with unique and temporally variable composition for each water supply (Schwarzenbach *et al.*, 2003). HS in soils and sediments can be divided into three fractions, humic acids (HA), fulvic acids (FA), and humin. HA and FA are extractable using a strong base, while humin cannot be extracted with water under basic or acidic conditions. Thus, water contains only HA and FA. HA are insoluble at low pH (≤1) while FA remain soluble. HS are typically amphiphilic and anionic at near neutral pH due to ionization of carboxylic acid groups (Owen *et al.*, 1995).

1.1.2.3 Performance

The removal of pathogens, organic matter, and various organic chemicals are documented for SRBF processes. Reported removals for viruses, bacteria, and protozoa in SSF processes are 2 to 4 log, 3 to 4 log, and 2 to 4 log, respectively, as summarized by Logsdon *et al*. (2002). Not surprising given longer bed lengths (and often slower water velocities), greater removals of particle contaminants are reported for RBF processes (Bouwer, *et al*., 1999; Wang, *et al*., 2002). The removal of dissolved organic matter and

disinfection byproduct precursors (*i.e*., trihalomethane and haloacetic acid formation potential) in SSF is typically less than 40% (Collins *et al*. 1989; Eighmy *et al*., 1993), with better performance (40 to 82 % removal) exhibitied by RBF (Bouwer *et al*., 1999; Grischek, 1999; Wang *et al*., 2002).

The performance of SSF with organic chemicals is varied, with the following removals reported: aromatics (*e.g.*, benzoate) <50%, chlorinated benzenes (<41%), polycyclic aromatic compounds (~85%), and ~25% for phenolic compounds (Haberer *et al*., 1984; Collins, 1989). Attributed to longer residence times and the presence of sequential oxic and anoxic regions, removal of organic compounds in RBF systems are typically greater than for SSF, with: complete removal of unsubstituted aromatic amines and limited removal of chlorinated anilines and nitroanilines, complete (below detect) and 70% removal of tri- and tetrachloroethylene, respectively (Bouwer *et al*., 1999; Grischek 1999; Wang, *et al*., 2002; Worch, *et al*. 2002). Recent attention has begun to focus on the fate of pharmaceutical compounds (*e.g.*, non-steroidal anti-inflammatory drugs, hormones, blood lipid regulators, and antibiotics in SRBF processes; with varied results depending on systems configuration, redox conditions, and the compound properties (Ying, *et al*., 2003 Rooklidge, 2004; Grünheid *et al*., 2005; Massmann *et al*., 2008).

1.1.3 Antibiotic Fate in SRBF Processes

Antibiotic fate in SRBF systems is dictated by abiotic or biotic transformation mechanisms (Figure 1.2). Although β-lactams are subject to hydrolysis (Hou and Poole, 1969; Volmer and Hui, 1998), and photolysis is reported for tetracyclines, quinolones,

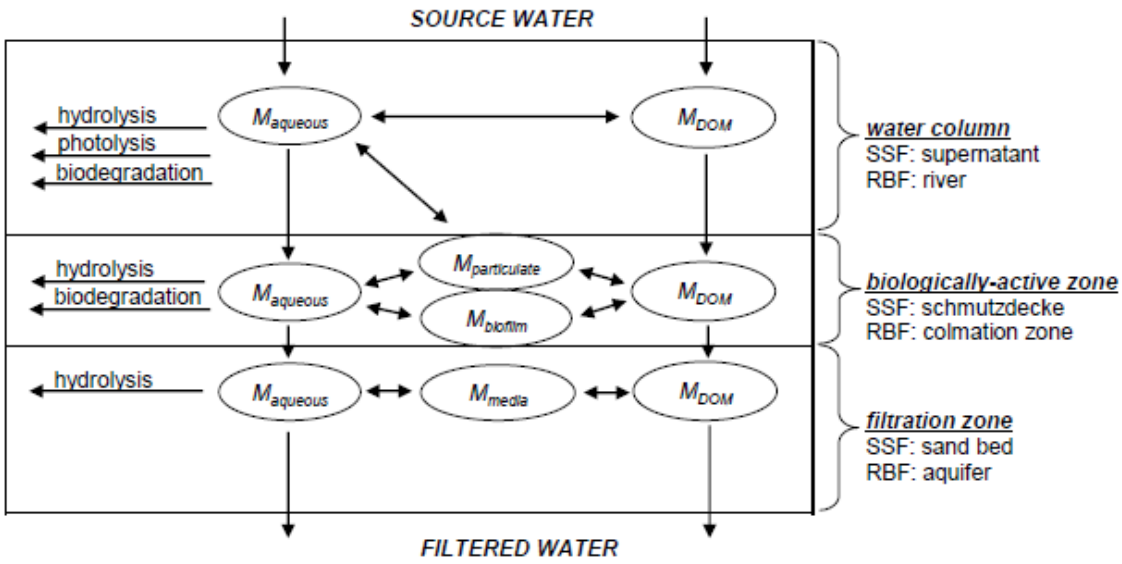


Figure 1.2. Antibiotic Fate in SRBF Systems. $M_{aqueous}$ is aqueous antibiotic, M_{DOM} is antibiotic partitioned to dissolved organic matter, $M_{particulate}$ is antibiotic sorbed to particulate matter, $M_{biofilm}$ is antibiotic sorbed to biofilm, and M_{media} is antibiotic sorbed to filter media (*i.e.*, sand or aquifer material), and (↔) represents antibiotic sorption/desorption

and sulfonamides in low turbidity circum-neutral water (Davies *et al.*, 1979; Moore and Zhou, 1994; Burhenne, *et al*, 1997; Tourniainen *et al.*, 1997), the operative loss mechanisms for antibiotics in SRBF processes are sorption and biodegradation.

1.1.3.1 Sorption

The sorption of organic chemicals in SRBF processes depends on the presence of organic carbon within the system. The sorption of antibiotics to organic carbon can be generally categorized by compound class. Although no research is available, β-lactams are not expected to readily sorb due to polarity and carboxylic acid functional groups that are ionized at near-neutral pH. Organic carbon partitioning coefficient values (K_{oc}) between 100 to 1000 L/kg have been reported for sulfonamides (Tolls *et al.*, 2002; Thiele-Bruhn

et at., 2004). Macrolide K_{oc} values generally range between 1000-10,000 L/kg, with tetracycline, quinoline, and aminoglycoside antibiotics exhibiting K_{oc} values between 10,000 to 100,000 L/kg (Yeager and Halley, 1990; Nowara *et al.*, 1997; Halling-Sorensen, 2000; Rabolle and Spliid, 2000; Loke *et al.*, 2002). Organic carbon sorption coefficients for antibiotics to biofilm is not reported in the literature.

1.1.3.2 Biodegradation

Reports of antibiotic biodegradation in various environmental matrices yield mixed results. Studying antibiotics at mg/L concentrations in soil inoculated with activated sludge bacteria, Gavalchin and Katz (1994) reported that erythromycin (ERY) is slowly biodegradable ($t_{1/2}$ of 11.5 days), while sulfamethoxazole (SMX) was recalcitrant. In 40-day closed-bottle tests of wastewater treatment plant effluent, biodegradation was not evident for SMX and ERY at mg/L concentrations (Alexy *et al*., 2004). Although SMX (at low mg/L concentrations) persisted over six-weeks in closed bottle tests with activated sludge (Al-Ahmad *et al*., 1999), biodegradation half-lives of 25.6 hours (20 µg/L in river sediment) and 64.2 hours (100 µg/L in activated sludge) were reported (Radke *et al*., 2009; Li and Zhang, 2010). Ingerslev and Halling-Sorensen (2000) noted that with microbial acclimation, SMX is aerobically biodegraded ($t_{1/2}$ of 0.2 to 3 days), while Drillia *et al.* (2005) report that biodegradation (at mg/L concentrations) only occurs in the absence of another carbon and nitrogen source for SMX. Highlighting the importance of redox conditions, Suarez *et al*. (2010) reported ERY removals due to biodegradation of 89 and 20% (at ~20 µg/L concentrations) under oxic and anoxic conditions, respectively, with SMX only removed (22%) under oxic conditions. Reports on ciprofloxacin (CIP)

biodegradation have been also mixed; with a half-life of ≥2 days reported for 250 µg/L concentrations in activated sludge (Rabolle and Splid, 2000; Halling-Sorensen *et al*., 2000), while others conclude that CIP is not biodegradable in activated sludge or biosolids (at 100 µg/L), and inhibitory to wastewater bacteria at concentrations as low 80 µg/L (Al-Ahmad *et al*., 1999; Wu *et al*., 2009; Li and Zhang, 2010).

1.1.4 Antibiotic Impacts on Biofilm Bacteria

Bacteria have many potential responses when exposed to antibiotics at sub-inhibitory levels. They can develop resistance to antibiotics when exposed over time to sub-inhibitory concentrations (Ash *et al.*, 2002; Esiobu, *et al*, 2002; Onan and LaPara, 2003). Also, the function and structure of bacterial communities can be altered by antibiotics at low (*i.e*., sub-inhibitory) concentration. For example, at concentrations about 3 orders-of-magnitude greater than those typically observed in aquatic systems (*i.e*., >1 mg/L), but below inhibitory levels, erythromycin, clarithromycin, and amoxicillin each reduced denitrification rates of bacteria in marine sediment (Costanzo *et al*., 2005).

There is some evidence that antibiotics at environmentally-relevant (*i.e*., low or sub-µg/L) concentrations may have adverse effects on bacteria. For example, changes in the morphology and quantity of colonies were observed for activated sludge bacteria exposed to various antibiotics at low µg/L concentrations (Alexy *et al*., 2004). Furthermore, soil bacteria respiration rate were inhibited when exposed to tetracycline at environmentally-relevant concentrations, while various sulfonamide antibiotics had little effect (Thiele-Bruhn, 2005). Little is known about the effects of antibiotic mixtures at low

concentrations on bacteria. Ghosh *et al*. (2009) reported that a mixture of antibiotics (clarithromycin, enrofloxacin, sulfamethoxazole, tetracycline and trimethoprim) at 50 μg/L (each compound) inhibited nitrifying bacteria. Furthermore, there is some evidence from the medical literature that mixtures of antibiotics at sub-inhibitory concentrations inhibit single-species bacterial biofilms has been reported in the medical literature (Neu, 1991; Monzón *et al*., 2001; Černohorská and Voltava, 2008). Consequently, there is a need to better understand the impact of antibiotic mixtures at environmentally-relevant concentrations on the function and structure of SRBF biofilm bacteria.

1.2 Scope of Dissertation

The goals of this research were to elucidate the fate of antibiotics in SRBF processes and to evaluate the impact of antibiotics on the biofilm bacteria and on process performance. The specific objectives of this research were to:

1. Assess the impact of mixtures of antibiotics at environmentally relevant concentrations on the activity and community structure of biofilm bacteria.

2. Quantify the kinetics and equilibrium of antibiotic sorption to biofilm.

3. Investigate the attenuation and effect of antibiotics on SRBF processes.

These objectives are addressed in Chapters 2, 3 and 4, respectively.

This dissertation is written in journal paper format, meaning that Chapters 2, 3 and 4 constitute individual journal articles. Three antibiotics, SMX, ERY, and CIP were selected for this work. Chapter 2 presents the results of experiments performed using continuous-flow rotating annular bioreactors (CFRAB) to assess whether low or sub-

μg/L levels of selected antibiotics inhibit the acetate utilization rate of the biofilm. Also, automated ribosomal intergenic spacer analysis (ARISA) was used to assess the changes in biofilm community composition resulting from antibiotics exposure. Chapter 3 discusses the results of experiments conducted using a CFRAB to quantify the rate and extent of sorption of selected antibiotics to multi-species biofilm. Chapter 4 concerns the attenuation and effect of selected antibiotics in SRBF processes. Lab-sclae slow sand filter experiments were performed to elucidate the fate of antibiotics during the start-up or maturation phase. Furthermore, ARISA was employed to assess the changes in biofilm community composition resulting exposure to the antibiotics at low or sub-μg/L levels. Finally, conclusions and future research needs are discussed in Chapter 5.

1.3 References

Adams, C., Wang, Y., Loftin, K., Meyer, M. (2002) Removal of antibiotics from surface and distilled water in conventional water treatment processes, Journal of Environmental Engineering 128(3), 253-260.

Al-Ahmad, A., Daschner, F.D., Kummerer, K. (1999) Biodegradability of Ceftiam, Ciprofloxacin, Meropenem, Penicillin G, and Sulfamethooxazole and inhibition of waste water bacteria. Archive of Environmental Contamination and Toxicology 37(2), 158-163.

Alexy, R., Kümpel, T., Kümmerer, K. (2004) Assessment of degradation of 18 antibiotics in the Closed Bottle Test. Chemosphere 57(6), 505-512.

Armstrong, J.L., Shigeno, D.S., Calomiris, J.J., Seidler, R.J. (1981) Antibiotic-resistant bacteria in drinking water. Applied Environmental Microbiology 42(2), 277-283.

Ash, R.J., Mauck, B., Morgan, M. (2002) Antibiotic resistance of gram negative bacteria in rivers, United States. Emerging Infectious Diseases 8(7), 713-716.

Benotti, M.J., Trenholm R.A., Vanderford B.J., Holady J.C., Stafford B.D., Snyder S.A. (2009) Pharmaceuticals and endocrine disrupting compounds in US drinking water. Environmental Science &Technology 43(9), 597-603.

Bouwer, E., Weiss, J., Ball, W., O'Melia, C., Arora, H. (1999) Water quality improvements during riverbank filtration at three Midwest utilities. Proc. International Riverbank Filtration Conference, Louisville, KY, USA.

Bouwer, E.J. (2003) Riverbank Filtration: The American Experience. In proc. 2nd International Riverbank Filtration Conference, Cincinnati, OH, USA.

Bryers, J. *Biofilms II-Process Analysis and Applications*. (2000) Wiley, New York, NY.

Burhenne, J., Ludwig, M., Spiteller, M. (1997) Photolytic degradation of fluoroquinolone carboxylic acids in aqueous solution. 1. Primary photoproducts and half-lives. Environmental Science and Pollution Research 4(1), 10-15.

Calamari, D., Zuccato, E., Castigliono, S., Bagnati, R., Fanelli, R. (2003) Strategic survey of therapeutic drugs in the rivers Po and Lambro in northern Italy. Environmental Science and Technology 37(7), 1241-1248.

Černohorská, L., Voltava, M. (2008) Antibiotic Synergy against biofilm-forming *Pseudomonas aeruginosa*. Folia Microbiology 53(1), 57–60.

Chamberlain, E., Adams, C. (2006) Oxidation of sulfonamides, macrolides, and carbadox with free chlorine and monochloramine. Water Research 40(13), 2517-2526.

Cleasby, J.L., Haarhoff, J. (1991) Biological and Physical Mechanisms in Slow Sand Filtration. *In* Slow Sand Filtration. Edited by G. Logsdon. ASCE Reston, VA.

Collins, M.R., Eighmy, T.T., Fenstermacher, J.M., Spanos, S.K (1989) Modifications to the Slow Sand Filtration Process For Improved Removals of Trihalomethane Precursors. AWWARF, Denver, CO.

Costanzo, S.D., Murby, J., Bates, J. (2005) Ecosystem response to antibiotics entering the aquatic environment. Marine Pollution Journal 51(1-4), 218-223.

Davies, A.K., McKellar, J.F., Phillips, G.O.; Reid, A.G. (1979) Photochemical oxidation of tetracycline in aqueous solution. Journal of Chemical Society-Perkin Trans. II 1979(3), 369-375.

Dodd, M.C., Huang, C-H. (2004) Transformation of the antibacterial agent sulfamethoxazole in reactions with chlorine: kinetics, mechanisms, and pathways. Environmental Science & Technology 38(21), 5607-5615.

Drillia, P., Dokianakis S.N., Fountoulakis M.S., Kornros M., Stamatelatou K., Lyberatos G. (2005) On the occasional biodegradation of pharmaceuticals in the activated sludge process: The example of the antibiotic sulfamethoxazole. Journal of Hazardous Materials 122(3), 259- 265.

Eighmy, T.T., Collins, M.R., Malley, J.P., Royce, J., Morgan, D. (1993) Biologically Enhanced Slow Sand Filtration for Removal of Natural Organic Matter. AWWARF, Denver, CO.

Esiobu, N., Armenta, L., Ike, J. (2002) Antibiotic resistance in soil and water environments. International Journal of Environmental Health Research 12(2), 133-144.

Flaherty, C.M. Dodson, S.I. (2005) Effects of pharmaceuticals on *Daphnia* survival, growth, and reproduction. Chemosphere 61(2), 200-207.

Flemming, C.A., Palmer, R.J., Arrage, A.A., Van der Mei, H.C., White, D.C. (1998) Cell surface physicochemistry alters biofilm development of Pseudomonas aeruginosa lipopolysaccharide mutants. Biofouling 13(3), 213-231.

Gavalchin, J., Katz, S.E. (1994) The persistence of fecal-borne antibiotics in soil. Journal of the Association of Official Analytical Communities International 77, 481-485.

Giger, W., Alder, A.C., Golet, E.M., Kohler, H.P.E., McArdell, C.S., Molnar, E., Siegrist, H., and Suter, M.J.F. (2003) Occurrence and fate of antibiotics as trace contaminants in wastewaters, sewage sludges, and surface waters. Chimia 57(9), 485-491.

Ghosh, G.C., Okuda, T., Yamashita, N. (2009) Occurrence and elimination of antibiotics at four sewage treatment plants in Japan and their effects on bacterial ammonia oxidation. Water Science and Technology 59(4), 779-786.

Giger, W., Alder, A.C., Golet, E.M., Kohler, H.P.E., McArdell, C.S., Molnar, E., Siegrist, H., and Suter, M.J.F. (2003) Occurrence and fate of antibiotics as trace contaminants in wastewaters, sewage sludges, and surface waters. Chimia 57(9), 485-491.

Grischek, T. (1999) Field Studies into the Attenuation of Contaminants During Riverbank Filtration. In. proc. International Riverbank Filtration Processes. Louisville, KY.

Grünheid, S., Amy, G., Jekel, M.(2005) Removal of bulk dissolved organic carbon (DOC) and trace organic compounds by bank filtration and artificial recharge. Water Research 39(14) 3219–3228.

Haberer, K.; Drews, M; Waltenberg, M. (1984) Entfernung organischer Wasserinhaltstoffe bei der Langsamsandfiltration. Vom Wasser 63, 121-140.

Halling-Sorensen, B. (2000) Algal toxicity of antimicrobial agents used intensive farming. Chemosphere 40(7), 731-739.

Hirsch, R., Ternes, T., Haberer, K., Kratz, K.L. (1999) Occurrence of antibiotics in the aquatic environment. Science of the Total Environment 225(1-2):109-118.

Hiscock, K.M., Grischek, T (2002) Attenuation of groundwater pollution by bank filtration. Journal of Hydrology, 266(3-4), 139-144.

Hokanson, D.R., Zhang, Q., Cowden, JR., Troschinetz, A.M., Mihelcic, J.R., Johnson, D.M. (2007) Challenges to implementing drinking water technologies in developing world countries. Environmental Engineer: Applied Research & Practice 1, 2-9.

Hou, J.P., Poole, J.W. (1969) Kinetics and mechanisms of degradation of ampicillan in solution. Journal of Pharmaceutical Sciences 58(4): 447-454.

Huang, C.H., Renew, J.E., Smeby, K.L., Pinkston, K., Sedlak, D.L. (2001) Assessment of potential antibiotic contaminants in water and preliminary occurrence analysis, proceedings of the 2nd International Conference on Pharmaceuticals and Endocrine Disrupting Chemicals in Water, National Ground Water Association, Minneapolis, MN.

Huber, M. M., Korhonen, S., Ternes, T. A., von Gunten, U. (2005) Oxidation of pharmaceuticals during water treatment with chlorine dioxide. Water Research 39(15), 3607-3617.

Ingerslev, F., Halling-Sørensen, B. (2000) Biodegradability properties of sulfonamides in activated sludge. Environmental Toxicology and Chemistry 19(10), 2467-2473.

Jahn, A., Nielsen P.H. (1995) Extraction of extracellular polymeric substances (EPS) from biofilms using a cation exchange resin. Water Science & Technology 32(8), 157-164.

Jekel, M., Grischek, T. (2003) Riverbank Filtration: The European Experience. In proc. 2nd Annual Riverbank Filtration Conference, Cincinnati, OH, USA

Kawamura S. (1991) *Integrated Design of Water Treatment Facilities*. John Wiley and Sons, New York, NY, USA.

Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., Buxton, H.T. (2002) Pharmaceuticals, hormones, and other organic wastewater constituents in US streams, 1999-2000: a national reconnaissance. Environmental Science and Technology 36(6), 1202-1211.

Kuehn W, Mueller U. (2000) Riverbank filtration: an overview. Journal AWWA 92(12), 60-69.

Lee, S.M., Davis, A.P. (2001) Removal of Cu (II) and Cd (II) from aqueous solution by seafood processing waste. Water Research 35(2), 534-540.

Li, B., Zhang, T. (2010) Biodegradation and Adsorption of Antibiotics in the Activated Sludge Process. Environmental Science & Technology 44(9), 3468-3473.

Liu, H., Fang, H.P. (2002) Characterization of electrostatic binding sites of extracellular polymers by linear programming analysis of titration data. Biotechnology and Bioengineering 80(7), 806-811.

Logsdon, G.S., ed. (1991) *Slow Sand Filtration* ASCE Environmental Engineering Water Supply Committee. ASCE, Reston, VA, USA.

Loke, M.L., Tjornelund, J., Halling-Sorensen, B. (2002) Determination of the distribution coefficient (log K_d) of oxytetracycline, tylosin A, olaquindox and metronidazole in manure. Chemosphere, 2002. 48(3), 351-361.

Massmann, G., Dünnbier, U., Heberer, T., Taute, T. (2008) Behaviour and redox sensitivity of pharmaceutical residues during bank filtration – Investigation of residues of phenazone-type analgesis. Chemosphere 71(8), 1476-1485.

McArdell, C.S., Molnar, E., Suter, M.J., Giger, W. (2003) Occurrence and fate of macrolide antibiotics in wastewater treatment plants and in the Glatt Valley watershed, Switzerland. Environmental Science and Technology 37(24), 5479-5486.

Miao, S.X., Bishay, F., Chen, M., Metcalfe, C.D. (2004) Occurrence of antimicrobial in the final effluents of wastewater treatment plants in Canada. Environmental Science and Technology 38(13), 3533-3541.

Monzón, M.; Oteiza, C.; Leiva, J, Amorena, B. (2001) Synergy of different antibiotic combinations in biofilms of *Staphylococcus epidermidis*. Journal of Antimicrobial Chemotherapy 48, 793-801.

Moore, D.E., Zhou, W. (1994) Photodegradation of sulfamethoxazole. A chemical system capable of monitoring seasonal changes in UVB intensity. Photochemistry and Photobiology 59(5), 497-502.

Neu, H.C. (1991) Synergy and antagonism of combinations with quinolones. European Journal of Microbiology and Infectious Diseases 10(4), 255-261.

Ngheim, L.D., Schäfer, A.I., and Elimelech, M. (2005) Pharmaceutical retention mechanisms by nanofiltration membranes. Environmental Science & Technology 39(19), 7698-7705.

Nowara, A., Burhenne, J., Spiteller, M. (1997) Binding of fluoroquinolone carboxylic acid derivatives to clay minerals. Journal of Agricultural Food Chemistry 45(4):1459-1463.

Onan, L.J., T.M. LaPara. (2003) Tylosin-resistant bacteria cultivated from agricultural soil. FEMS Microbiology Letters 220(1):15-20.

O'Shea R., Moser, H.E. (2008) Physicochemical properties of antibacterial compounds: implications for drug discovery. Journal of Medicinal Chemistry 51(10), 2871-2878.

Owen, D.M, Amy, G.L., Chowdhury, Z.K., Paode, R., McCoy, G., Viscosil, K. (1995) NOM characterization and treatability. Journal AWWA 87(1), 46-63.

Prankerd, R.J. (2007) *Profiles of Drug Substances, Excipients and Related Methodology, Volume 33: Critical Compilation of pKa Values for Pharmaceutical Substances (Analytical, Excipients, and Related Methodology)* Academic Press, Amsterdam, Netherlands

Qiang, Z., Adams, C. (2004) Potentiometric determination of acid dissociation constants (pK_a) for human and veterinary antibiotics. Water Research 38(12), 2874-2890.

Rabolle, M., Spliid, N.H. (2000) Sorption and mobility of metrondazole, olaquindox, oxytetracycline and tylosin in soil. Chemosphere 40(7), 715-722.

Rachwal, A.J., Bauer, M.J., Chipps, J.S., Colbourne, J.S., Foster, D.M. (1996) Comparisons between slow sand and high rate biofiltration. In proceedings: Third International Slow Sand/Alternative Biofiltration Conference. John Wiley and Sons, NY, USA.

Radke, M., Lauwigi, C., Heinkele, G., Murdter, T.E., Letzel, M. (2009) Fate of the antibiotic sulfamethoxazole and its two major human metabolites in a mater sediment test. Environmental Science and Technology 43 (9), 3135-3141.

Rooklidge, S.J. (2004). Antimicrobial Contaminant Removal by Mulit-stage Drinking Water Filtration. PhD Dissertation, Oregon State University, Corvallis, OR.

Schwarzenbach, R.P., Gschwend, P.M., Imboden, D. (2003) *Environmental Organic Chemistry, 2nd Edition*, Wiley Interscience, New Jersey.

Schwartz, T., Kohnen, W., Jansen, B., Obst, U. (2003) Detection of antibiotic-resistant bacteria and their resistance genes in wastewater, surface water, and drinking water biofilms. FEMS Microbiology Ecology 43(3), 325-335.

Snyder, S.A., Adham, S., Redding, A.M., Cannon, F.S., DeCarolis, J., Oppenheimer, J., Yoon, Y. (2007) Role of membranes and activated carbon in the removal of endocrine disruptors and pharmaceuticals. Desalination 202(1-3), 156-181.

Suarez, S., Lema, J.M., Omil, F. (2010) Removal of pharmaceutical and personal care products (PPCPs) under nitrifying and denitrifying conditions. Water Research 44(10), 3214-3224.

Takács-Novák, K., Noszál, B., Tokes-Kovesdi, M., Szasz, G., (1993) Acid base properties and proton-speciation of vancomycin. International Journal of Pharmacy89(3), 261-263.

Thiele-Bruhn, S., Seibicke, T., Schulten, H.R., Leinweber, P. (2004) Sorption of sulfonamide pharmaceutical antibiotics on whole soils and particle-size fractions. Journal of Environmental Quality 33(4), 1331-1342.

Thiele-Bruhn, S. (2005) Microbial inhibition by pharmaceutical antibiotics in different soils—dose-response relations determined with the iron (III) reduction test. Environmental Toxicology and Chemistry 24(4), 869-876.

Tolls, J., Gebbink, W., Cavallo, R. (2002) pH-dependence of sulfonamide antibiotic sorption: data and model and evaluation. In proceedings: SETAC Europe 12th Annual Meeting, Vienna.

Torniainen, K., Mattinen, J., Askolin, C.P., Tammilehto, S. (1997) Structure elucidation of a photodegradation product of ciprofloxacin. Journal of Pharmaceutical and Biomedical Analysis 15(7), 887-894.

Volmer, D.A., Hui, J.P.M. (1998) Study of erythromycin A decomposition products in aqueous solution by solid-phase microextraction/liquid chromatography/tandem mass spectrometry. Rapid Communications in Mass Spectrophotometry 12(3),123-129.

Wang, J, Hubbs, S., Song, R. (2002) Evaluation of Riverbank Filtration as a Drinking Water Treatment Process. AWWARF. Denver, CO.

Weber-Shirk, M., Dick, R.I. (1997) Physical-chemical mechanisms in slow sand filters. Journal AWWA 89(1), 87-100.

Westerhoff, P., Yoon, Y., Snyder, S., Wert, E. (2005) Fate of endocrine-disruptor, pharmaceutical, and personal care product chemicals during simulated drinking water treatment processes. Environmental Science & Technology, 39(17), 6649-6663.

Wiegel, S., Aulinger, A., Brockmeyer, R., Harms, H., Löffler, J., Reincke, H. Schmidt, R.Stachel, B., von Tümpling, W., Wanke, A. (2004) Pharmaceuticals in the river Elbe and its tributaries. Chemosphere 57(2), 107-126.

Worch, E., Grischek, T., Boernick, H., Eppinger, P. (2002) Laboratory tests for simulating attenuation processes of aromatic amines in riverbank filtration. Journal of Hydrology 266(3-4): 259-268.

Wu, C., Spongberg, A.L., Witter, J.D. (2009) Sorption and biodegradation of selected antibiotics in biosolids. Journal of Environmental Science and Health Part A 44(5), 454-461.

Yang, Li-H., Ying, G-G., Su, H.C., Stauber, J.L., Adams, M.S., Binet, M.T. (2008) Growth-inhibiting effects of 12 antibacterial agents and their mixtures on the freshwater microalga *Pseudokirchneriella subcapitata*. Environmental Toxicology and Chemistry 27(5), 1201–1208.

Yeager, R.L., Halley, B.A. (1990) Sorption/Desorption of [^{14}C]efrotymycin with soils. Journal of Agricultural Food Chemistry 38(3), 883-886.

Ying, G-G, Kookana, R.S., Dillion, P. (2003) Sorption and degradation of selected five endocrine disrupting chemicals in aquifer material. Water Research 37(15)3785-3791.

Chapter 2. Effect of Mixtures of Antibiotics on the Structure and Function of Bacterial Biofilm Communities

Antibiotics are present in surface waters worldwide, but little is known about the effects of these compounds on the biological processes (*e.g.*, slow rate filtration, bank filtration) often used to treat water. In this research, the effect of antibiotics on bacterial activity and community structure was investigated by growing biofilms in the presence and absence of a mixture of three compounds (sulfamethoxazole (SMX), erythromycin (ERY), and ciprofloxacin (CIP)) in a continuous-flow rotating annular bioreactor and fed acetate as substrate. Steady-state areal substrate utilization rates for all antibiotic treatments (all at 0.33 μg/L, all at 3.33 μg/L, and 1 at 3.33 μg/L with the other 2 at 0.33 μg/L) were similar to the control experiments, and utilization rates did not change during an antibiotic shock loading experiment (all at 3.33 μg/L). Greater attached biomass levels in the experiments with CIP at 3.33 μg/L resulted in lower steady-state biomass-normalized substrate utilization rates in comparison to all of the other runs. Also, microbial community analyses via automated ribosomal intergenic spacer analysis (ARISA) revealed shifts in community structure for the high dose CIP experiments. Thus, the biofilm responded to the high dose CIP by shifting community structure, presumably to more resistant bacterial strains, and by producing more biomass. Overall, the results of this research suggest that mixtures of antibiotics at the sub-μg/L concentrations typically observed in surface waters are unlikely to affect biological process performance.

2.1 Introduction

Antibiotics have been detected in water supplies world-wide at concentrations of up to 1.9 μg/L, but typically in the low ng/L range (Kolpin *et al*., 2002). One source of these compounds is treated wastewater, where concentrations of up to 1.8 μg/L have been reported (Renew and Huang, 2004). Another potential source is agricultural operations, as antibiotics often are given to farm animals for growth promotion and prophylaxis (Mellon *et al.*, 2001; Boxall *et al*., 2003). Antibiotics are generally categorized according to their basic structure and mechanism of action (O'Shea and Moser, 2008). β-lactam antibiotics inhibit cell wall (*i.e*. peptidoglycan) synthesis and numerous antibiotic classes inhibit protein synthesis (*e.g.*, macrolides, aminoglycosides, and tetracyclines). Sulfonamides inhibit folic acid production and fluoroquinolones inhibit the production of DNA gyrase (*i.e*., topoisomerase II). There is increasing concern over the presence of pharmaceutical compounds, including antibiotics, in drinking water (Benotti *et al*., 2009). Another public health concern regarding the occurrence of antibiotics in aquatic systems is the development of antibiotic-resistant bacteria (Ash *et al*., 2002).

Biofiltration systems involve passing water (or air) through a bed of natural or engineered support media (*e.g.*, sand, aquifer material, or granular activated carbon) that has been colonized by bacteria residing in biofilms. There are numerous operational configurations, generally categorized by filtration rate or application, including: rapid (bio)filtration (5 to 25 m/hr), slow sand filtration (~0.1 m/hr), bank filtration (<0.1 m/hr), and aquifer storage and recovery (Kawamura, 1995; Kuehn and Mueller, 2000; Snyder *et al*., 2007). Biofiltration systems remove pathogens and organic compounds such as

disinfection byproduct precursors, pesticides, and pharmaceutical compounds (Weber-Shirk and Dick, 1997, Collins *et al.*, 1999, Hiscock and Grischek, 2002; Heberer *et al*, 2004). In addition, there is recent evidence that antibiotics can be removed in slow-rate biofiltration systems (Rooklidge, 2005; Heberer *et al*., 2008).

Although biofiltration systems may be effective at removing antibiotics from water supplies, the bacteria that are critical for treatment effectiveness might be adversely affected by antibiotics in the source water. For example, at concentrations about 3 orders-of-magnitude greater than those typically observed in aquatic systems (*i.e*. >1 mg/L), erythromycin, clarithromycin, and amoxicillin each reduced denitrification rates of bacteria in marine sediment (Costanzo *et al*., 2005). There is also some evidence that single antibiotics at environmentally-relevant (*i.e*. low or sub-µg/L) concentrations may have detrimental effects on bacteria in aquatic and soil environments. For example, changes in the morphology and quantity of colonies were observed when activated sludge bacteria were plated before and after exposure to various antibiotics at low µg/L concentrations (Alexy *et al*., 2004). Furthermore, the respiration rate of soil bacteria was inhibited when exposed to tetracycline at environmentally-relevant concentrations. In contrast, various sulfonamide antibiotics had little effect (Thiele-Bruhn, 2005). Unfortunately, little research has been done to investigate the effects of mixtures of antibiotics at low concentrations. Ghosh *et al*. (2009) reported that a mixture of antibiotics (clarithromycin, enrofloxacin, sulfamethoxazole, tetracycline and trimethoprim) at concentrations (for each compound) of 50 µg/L inhibited nitrifying bacteria. Several studies from the medical literature have demonstrated the synergistic

effects of mixtures of antibiotics from different classes on bacterial biofilms even though each antibiotic was below its inhibitory concentration (Neu, 1991; Monzón *et al*., 2001; Černohorská and Voltava, 2008).

In this study, we report the results of laboratory experiments designed to investigate the effects of mixtures of antibiotics at environmentally-relevant concentrations on the activity and structure of bacterial communities in biofilms grown in a continuous-flow rotating annular bioreactor (CFRAB). Monod plots (*i.e*. acetate utilization rate as a function of acetate concentration) were developed to evaluate the effect of antibiotic mixtures on the activity of biofilm bacteria. Automated ribosomal intergenic spacer analysis (ARISA) was used to assess the effects of antibiotics on community structure.

2.2 Materials and Methods

2.2.1 Experimental Design

The CFRAB (Biosurface Technologies, Bozeman, MT, USA) was selected for this work. A schematic diagram of the experimental system is shown in Figure 2.1. Although the main motivation of our work is the potential inhibition of bacteria in biofiltration systems by mixtures of antibiotics at low concentrations, the CFRAB system was selected because the complete-mix system is easy to model (*i.e*., for determining kinetic parameters) and the removable slides facilitate collection of the biofilm for quantification and community analysis.

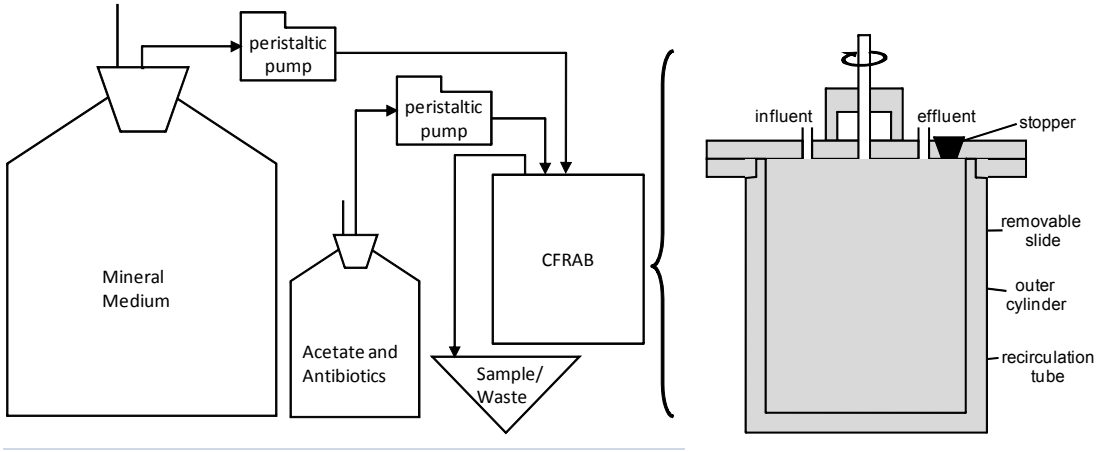
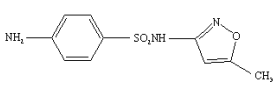
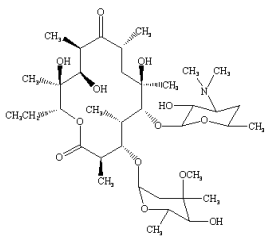
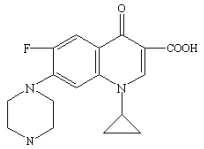


Figure 2.1. Schematic diagram of the continuous-flow rotating annular bioreactor system used for the experiments.

Three antibiotics (sulfamethoxazole (SMX), erythromycin (ERY), and ciprofloxacin (CIP)) were selected for these studies (Table 2.1) because they: (1) represent three prominent classes of antibiotics with different mechanisms of action, (2) have been detected in surface water, and (3) exhibit different physicochemical properties that would affect their interaction with biofilm.

Table 2.1. Characteristics of the Antibiotics Selected for Impact Experiments

Antibiotic	Sulfamethoxazole	Erythromycin	Ciprofloxacin
Class	sulfonamide	macrolide	fluoroquinolone
Mechanism of action	Folic acid production	Protein Synthesis (50S subunit of RNA)	DNA replication (topoismerase II)
Spectrum	Gram-positive and gram-negative	Gram-positive	Broad
Molecular weight	253.3	733.9	331.3
Molecular structure	 $C_{10}H_{11}N_3O_3S$	 $C_{37}H_{67}NO_{13}$	 $C_{17}H_{18}FN_3O_3$

Herein, all of these compounds are referred to as antibiotics even though SMX and CIP, as synthetic compounds, are not true antibiotics. SMX is a sulfonamide that has been detected in surface water and wastewater treatment plant (WWTP) effluents at concentrations up to 1.9 and 1.8 μg/L, respectively (Kolpin *et al*. 2002; Renew and Huang, 2004). ERY is a macrolide antibiotic that has been detected in surface waters and WWTP effluents at concentrations up to 1.7 and 0.81 μg/L, respectively (Kolpin *et al.*, 2002; Lin *et al.*, 2009). CIP is a fluoroquinolone antibiotic that has been detected in surface waters and WWTP effluents at concentrations up to 119 and 970 ng/L, respectively (Batt *et al*., 2006; Pena *et al*., 2007). Reported logK_{ow} values at pH 7.4 (25 °C) for SMX, ERY, and CIP are -0.9, 1.58, and -1.1, respectively (Hansch *et al*., 1995; Drakapoulos *et al*., 1997). Reported biofilm organic carbon partitioning coefficients (K_{oc}) for SMX, ERY, and CIP are 4,000, 6,000, and 92,000 L/kg respectively (Chapter 3).

Multiple experiments were run with varied concentrations and combinations of antibiotics as follows: (1) no antibiotics (control); (2) the three antibiotics each at 3.33 μg/L; (3) the three antibiotics each at 0.33 μg/L; (4) 3.33 μg/L CIP and 0.33 μg/L of both ERY and SMX; (5) 3.33 μg/L ERY and 0.33 μg/L of both CIP and SMX; and (6) 3.33 μg/L SMX and 0.33 μg/L of both ERY and CIP. All experiments were performed in duplicate except for conditions 5 and 6, which were only performed once.

2.2.2 Chemicals

Antibiotics (SMX, ≥98% purity; ERY, ≥95% purity; and CIP, ≥98% purity) were used to make aqueous stock solutions (prepared at least monthly) from which feed solutions were made daily. All antibiotics were purchased from Sigma Aldrich (St. Louis, MO, USA) and used as received.

Fresh mineral medium (pH 7.25 ± 0.3) was prepared (using distilled water) every four to five days and contained (per liter of reactor water): 43.8 mg/L K_2HPO_4, 17 mg/L KH_2PO_4, 62.4 mg/L Na_2HPO_4, 45.0 mg/L $MgSO_4$, 0.5 mg/L $FeCl_3$-$6H_2O$, 5.4 mg/L NH_4Cl, and 55 mg/L $CaCl_2$. The acetate feed solution was prepared daily by dissolving sodium acetate in distilled water. All salts used to prepare the mineral medium were reagent grade (Sigma Aldrich, St. Louis, MO).

2.2.3 CFRAB Operation

For each run, the CFRAB was autoclaved and then inoculated. The inoculum consisted of biomass cryopreserved in 15% glycerol (v/v) harvested from a previous CFRAB run in which the reactor was seeded with schmutzdecke from a slow sand filter at The Metropolitan District (MDC) in Hartford, CT and then fed acetate (2 mg C/L) for 2 weeks at a hydraulic residence time (HRT) of 30 minutes. A cryovial containing ~0.5 grams of biomass was thawed and the biomass was revived by culturing in a 2-L Erlenmyer flask containing 100 mg acetate as C in 1 L of mineral medium for 24 hours in the dark at room temperature (~24°C). Then, the inoculum solution was prepared by mixing 500 mL of the 24-hour culture with 500 mL of fresh mineral medium containing

100 mg acetate as C. The CFRAB was inoculated by transferring the inoculum solution into the reactor and then operating in batch mode (*i.e*. no flow) for 24 hours with mixing at 100 rpm. After inoculation, the mineral medium and acetate feed solutions (influent acetate concentration of 2 mg C/L) were initiated and the CFRAB was operated with a 30 minute HRT and 100 rpm mixing in the dark (*i.e*. covered with aluminum foil) at room temperature (~24°C). In experiments with antibiotics, the antibiotics were added to the acetate reservoir and introduced at the start of continuous-flow operation. The reactors were operated in continuous-flow mode for 14 days prior to beginning the acetate utilization kinetics experiments. Effluent acetate concentrations were below detection limits (25 μg/L) typically within 7 days of initiating continuous-flow operation and the CFRAB had effectively achieved steady state conditions.

2.2.4 Acetate Utilization Kinetics Experiments

At 14 days, an "acetate sweep" experiment was conducted for developing Monod curves (*i.e*. substrate utilization rate vs. substrate concentration) to obtain kinetic parameters. The acetate sweep experiment was performed by sequentially stepping up the acetate feed concentration (*i.e*. from 2 mg C/L initially to 6, 8, 10, and 12 mg C/L), holding for 60 minutes (2 HRTs), and then collecting effluent samples for acetate analysis. Three effluent samples were collected sequentially in 130 mL amber glass bottles spiked with the biocide benzalkonium chloride (30 mg/L) as a preservative. To check for aerobic conditions in the CFRAB, the dissolved oxygen (DO) was measured in the effluent for selected runs and was consistently greater than 5.0 mg/L. Immediately after collection of the three effluent samples, the samples were filtered (0.45 μm nylon syringe filters) into

sterile vials and stored in the refrigerator (~4°C) for up to 36 hours until the acetate analyses were performed. At the end of each run, biofilm was scraped from the CFRAB slides using slitted silicone tubing (Masterflex 96400-24) for immediate volatile solids analysis and subsamples were placed in 2 mL sterile cryovials and frozen at -70°C for subsequent DNA extraction and community analysis.

2.2.5 Antibiotic Shock Loading Experiments

Another experiment was conducted to investigate the response of biofilm bacteria to antibiotic shock loading. For this run, the CFRAB was inoculated and then operated without antibiotics in the feed for 14 days at which time the antibiotic feed (3.33 μg/L, each) was initiated. Then, acetate sweeps (with feed concentrations of 14, 18, 22, and 26 mg/L) were conducted at 2 hours, 2 days, and 18 days following the initiation of antibiotic feed. The feed water was supersaturated with DO (to ~25 mg/L) during the acetate sweeps to ensure that DO was not limiting and effluent DO levels were consistently greater than 7.5 mg/L.

2.2.6 Analytical Methods

2.2.6.1 Acetate

Acetate was analyzed via ion chromatography (IC) using a method adapted from Kuo (1998). Four point calibration curves were developed for each run at acetate concentrations ranging from 25 to 400 μg C/L, with correlation coefficient (R^2) values consistently greater than 0.995. Additional method details are provided by Appendix B.

2.2.6.2 Biomass as Volatile Solids

Biofilm was harvested from 15 polycarbonate slides by running the slide through slitted silicon tubing and transferring the biofilm to aluminum weighing trays. The biofilm sample was dried to a constant weight at 105 °C and then combusted at 550 °C for 2 hours. Volatile solids (VS) was computed as the difference between dry weight and post-combustion weight and then normalized by the total surface area of the slides. Total reactor VS was estimated by multiplying the average area-normalized mass per slide by the total surface area inside the reactor.

2.2.6.3 Community Structure via ARISA

DNA was extracted from the biofilm samples using a soil DNA isolation kit (MoBio UltraClean™) and the extracted DNA was stored at -70°C until further processing. The intergenic spacer regions were amplified via polymerase chain reaction (PCR) using primers ITSF and ITSReub (Cardinale *et al*., 2004); primer ITSF was labeled with 6-carboxyhexafluorescein (HEX) at the 5′-end. Amplified products were resolved by capillary electrophoresis (ABI 3130xl, Applied Biosystems Inc; Foster City, CA) at the University of Minnesota Biomedical Genomics Center. The length of the fragments was estimated using the MapMarker 1000 size standard (Bioventures; Murfeesboro, TN). Electropherograms were visualized using GeneMapper software, version 4.0 (Applied Biosystems). The peak detection limit was set at 150 relative fluorescence units. Peaks comprising less than 1% of the total peak area were excluded from the analysis. Additional details of the ARISA methodology are available in Nelson *et al*. (2010).

2.2.7 Data Analysis

2.2.7.1 Acetate Utilization Kinetics

Monod curves were developed for each run by determining acetate utilization rates at various reactor acetate concentrations. Acetate utilization rate (r, mg C/L/day) was determined from the difference between reactor influent and effluent acetate concentrations according to the following mass balance equation:

$$r = -\frac{Q(S_{\text{inf}} - S)}{V}$$

where: S_{inf} is the influent acetate concentration (mg C/L), S is the effluent acetate concentration (mg C/L), Q is the flowrate (L/d), and V is the liquid volume in the reactor (L). For each experiment, TableCurve2D version 5.01 software (Systat Software, Chicago, IL) was used to determine the biokinetic parameters by fitting the utilization rate data to the following equation:

$$r = -\frac{kS}{K_S + S}$$

where: k is the maximum substrate utilization rate (mg C/L/day) and K_s is the half-velocity constant (mg C/L). The surface area-normalized substrate utilization rate (r_{areal}, mg C/cm^2/day) and maximum substrate utilization rate (k_{areal}, mg C/cm^2/day) for each experiment were obtained by multiplying r and k, respectively, by the reactor volume (V = 1 L) and then dividing by the reactor surface area (A = 2960 cm^2). The biomass-

normalized rate constant (k_{vs}, mg C/mg VS/day) was obtained in a similar manner except that k was divided by VS instead of area.

2.3 Results

2.3.1 Acetate Utilization Kinetics

Exposure to low-level concentrations of mixed antibiotics had little effect on the kinetics of acetate utilization. Plots of areal substrate utilization rate (r_{areal}) versus reactor substrate concentration are provided in Figure 2.2. Correlation coefficients (R^2) for the regressions were greater than 0.9 for all runs with a mean of 0.948. The k_{areal} for the different conditions were similar and ranged from 0.12 ± 0.01 to 0.16 ± 0.01 mg C/cm^2/day (Table 2.2). Half-velocity coefficients (K_s) ranged from 0.0004 ± 0.0004 to 0.019 ± 0.019 mg C/L with 95% confidence intervals of up to 100% of the regressed values (Table 2.2). Biomass (as VS) values for the six runs with no CIP or low CIP (*i.e.*, 0.33 µg/L) were similar with a mean (± 95% confidence interval) of 244 ± 68 mg/L that was less than that for the four experiments with high CIP (352 ± 47 mg/L).

Table 2.2. Summary of the biokinetic parameters obtained from the Monod plots.

Condition	Reactor biomass (mg VS)[A]	Half-velocity coefficient, K_s (mg C/L)[A]	k_{areal} (mg C/cm2/day)[A]	k_{VS} (mg C/mg VS/day)[A]
Control (1)	260 ± 18	0.004 ± 0.001	0.15 ± 0.0006	1.72 ± 0.12
Control (2)	219 ± 20	0.0004 ± 0.0004	0.12 ± .006	1.64 ± 0.23
All @ 0.33 µg/L (1)	225 ± 21	0.007 ± 0.001	0.12 ± 0.009	1.60 ± 0.27
All @ 0.33 µg/L (2)	292 ± 49	0.007 ± 0.003	0.16 ± 0.01	1.69 ± 0.39
All @ 3.33µg/L (1)	308 ± 28	0.009 ± 0.001	0.13 ± 0.0001	1.26 ± 0.11
All @ 3.33µg/L (2)	275 ± 24	0.004 ± 0.003	0.13 ± 0.01	1.42 ± 0.23
CIP@3.33µg/L, ERY&SMX @ 0.33 µg/L (1)	390 ± 27	0.003 ± 0.001	0.12 ± 0.005	0.92 ± 0.10
CIP@3.33µg/L, ERY&SMX @ 0.33 µg/L (2)	434 ± 14	0.001 ± 0.001	0.14 ± 0.004	0.96 ± 0.06
ERY@3.33µg/L, SMX&CIP @ 0.33 µg/L	217 ± 18	0.019 ± 0.019	0.12 ± 0.01	1.66 ± 0.28
SMX@3.33µg/L, CIP&ERY @ 0.33 µg/L	252 ± 24	0.016 ± 0.007	0.15 ± 0.01	1.79 ± 0.29

[A]mean ± 95% confidence interval

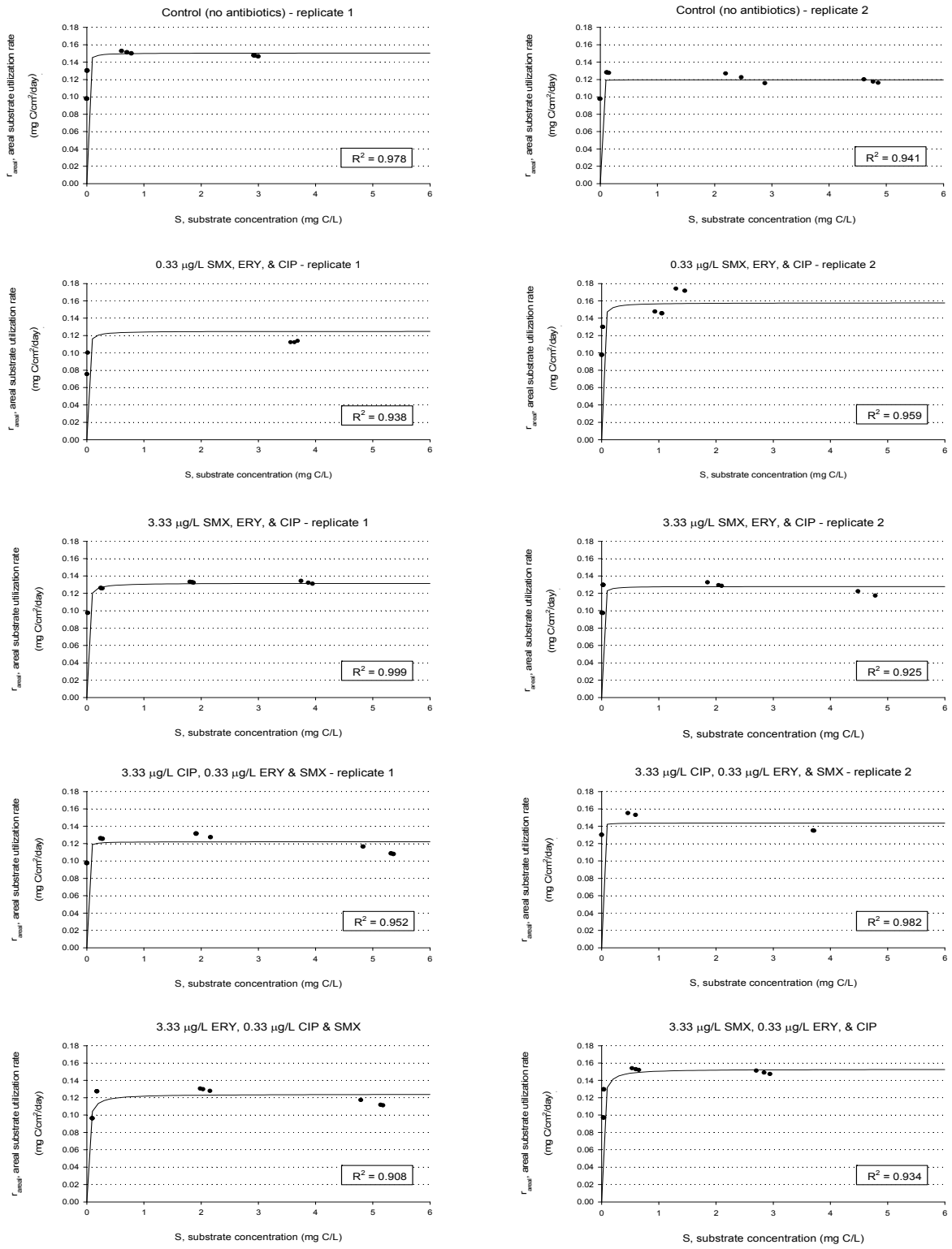


Figure 2.2. Monod plots showing the effect of antibiotic mixtures on areal acetate utilization rate (r_{areal}). Data points are experimental results and lines indicate best fit of the equation $r_{areal} = -k_{areal}S/(K_s+S)$. The correlation coefficient (R^2) values indicate the goodness of fit.

2.3.2 Antibiotic Shock Loading Experiment

The presence of low concentrations of antibiotics under shock-loading conditions did not affect the kinetics of acetate utilization. Multiple acetate sweeps were performed during the antibiotic shock loading experiment before and after the step input of antibiotics. The maximum areal substrate utilization rate for this experiment was 0.35 ± 0.01 before initiating antibiotic loading and after initiating antibiotic loading the rates were 0.38 ± 0.04 mg C/cm^2/day (time = 2 hours), 0.42 ± 0.01 mg C/cm^2/day (time = 2 days), and 0.42 ± 0.01 mg C/cm^2/day (time = 18 days). Because biomass was not quantified during the extended run, biomass-normalized utilization rates are not reported.

2.3.3 Community Structure

Low levels of antibiotic mixtures did not affect the bacterial community structure unless CIP was supplied at the "high" concentration of 3.33 μg/L. The community fingerprints from the ARISA analyses are summarized in Figure 2.3. The 769 bp fragment was a dominant fragment (≥40% of total peak area) for all of the experiments except those with high CIP. For example, the 769 bp fragment represented 92.6% and 61.0% of the total peak area for the two control reactors. Conversely, the 769 bp fragment was a minor component of the biofilm samples from the four high CIP experiments, ranging from 0 to 9.7% of the total peak area. The 757 bp fragment was the dominant fragment in both experiments with the three antibiotics fed at 3.33 μg/L (representing 42.5% and 53.6% of the total peak area), but this fragment was relatively minor otherwise (except for control run 2, 22.1%). When CIP was supplied at 3.33 μg/L and SMX and ERY were supplied at 0.33 μg/L, the bacterial community fingerprints were characterized by a greater number

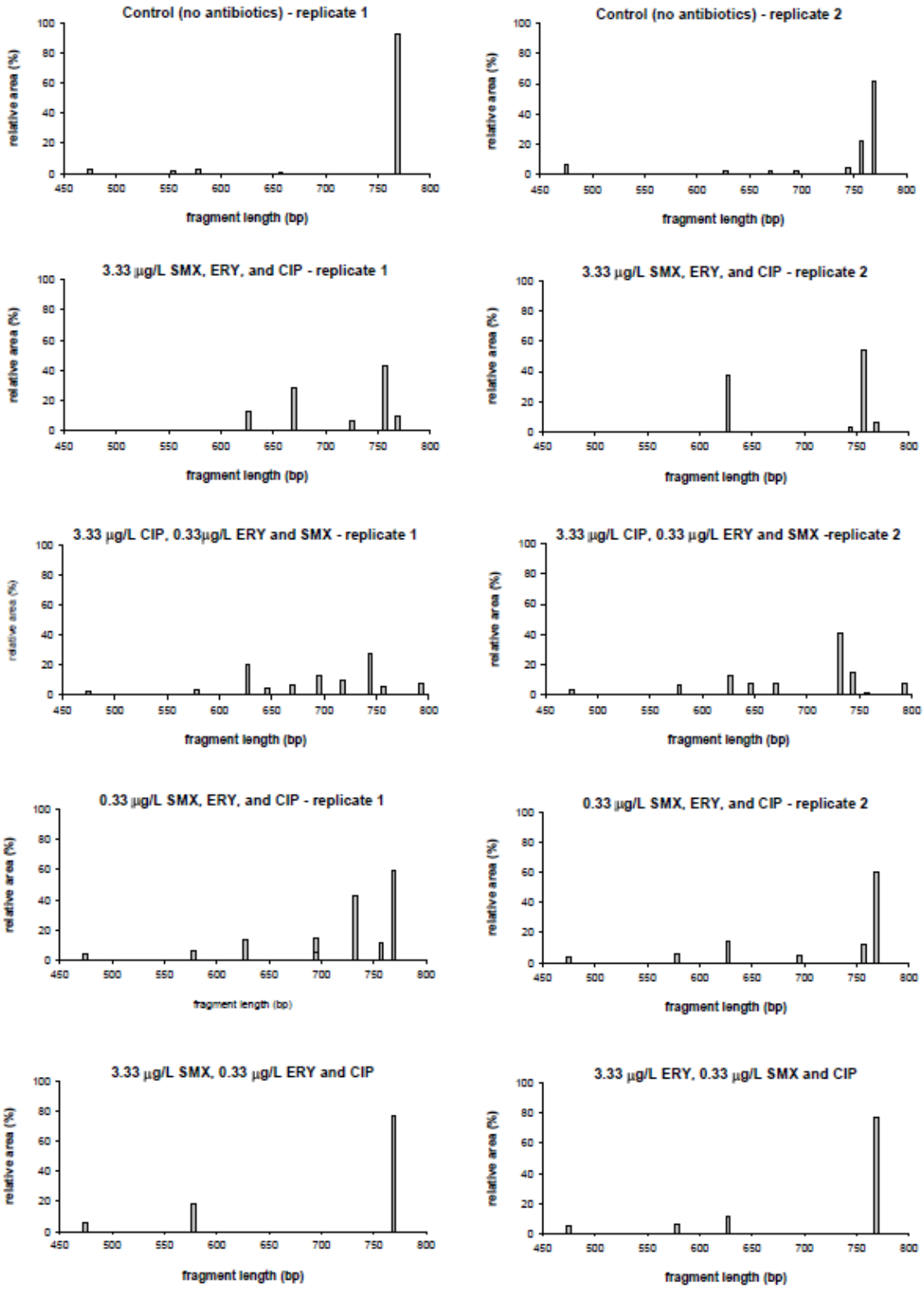


Figure 2.3. Effect of antibiotic mixtures on microbial community composition determined using ARISA.

number of fragments with most representing a relatively small fraction of the total peak area (*i.e.* ≤ 20%). The 627 bp peak (20.1% and 12.8%) and the 744 bp peak (27.1% and 14.8%) were major peaks in both of those experiments with the 732 bp peak dominant in run 2 (40.5%) but not showing up in the run 1 sample. The 793 bp fragment was only present (7.0 and 7.6 %) for this feed condition (*i.e.*, high CIP, low SMX and ERY).

2.4 Discussion

Surface area-normalized acetate utilization rates (k_{areal}) for reactors continuously fed mixtures of three antibiotics (ERY, SMX, and CIP), even at levels higher than expected in natural waters, were similar to control runs without antibiotics. Furthermore, shock loading of the three antibiotic mixture at a concentration of 3.33 μg/L for each antibiotic had no effect on areal acetate utilization rates. Thus, on the basis of our results we conclude that mixtures of antibiotics at environmentally relevant levels are unlikely to affect overall bacterial activity in biofiltration systems. Reported minimum inhibitory concentrations (MIC) for the three antibiotics (Lorian, 1996) are 3 to 15,000 times higher than the highest concentration used in this work (3.33 μg/L): ERY (0.25 mg/L for *Staphylococcus aureus* and 50 mg/L for *Escherichia coli*), SMX (32 mg/L for *S. aureus* and 16 mg/L for *E. coli*), and CIP (0.25 mg/L for *S. aureus* and 0.01 mg/L for *E. coli*). There is a substantial body of work from the medical community, however, demonstrating that mixtures of antibiotics, at doses considerably below what are required when administered singly, can be effective at combating infection (Acar, 2000; Cottarel and Wierzbowski, 2007). Even the relatively few studies in environmental settings demonstrated the adverse effects of mixtures of antibiotics at low concentrations on

bacterial activity. For example, a mixture of 12 antibiotics (each at 0.1 μg/L) inhibited the growth of the microalga *Pseudokirchneriella subcapitata* but not when dosed individually (Yang *et al*., 2008). Although nitrification of activated sludge effluent was not inhibited by individual antibiotics (clarithromycin, enrofloxacin, sulfamethoxazole, tetracycline and trimethoprim) at concentrations below 50 μg/L, a mixture of those antibiotics, each at 50 μg/L, decreased nitrification rates by 25% (Ghosh *et al*., 2009). These experiments differ from those described herein, however, in that Yang *et al*. (2008) dosed a larger number of antibiotics and targeted a single microbial species while the antibiotic concentrations in the Ghosh *et al*. (2009) study were much greater than those employed in our work.

Multi-species bacterial communities are typically more robust than single-species systems (Naeem and Li, 1997) and can adapt to toxic inputs by shifting community composition to more resistant strains (von Canstein *et al*., 2002). Indeed, although community function (*i.e*., acetate degradation) was not affected by a mixture of three antibiotics at sub-inhibitory concentrations, there was a clear shift in community structure when CIP was supplied at 3.33 μg/L regardless of the concentrations of ERY and SMX. Specifically, the organism represented by the 769 bp fragment was predominant (39.6 to 92.6% of total) in all experiments where CIP was at 0.33 μg/L or absent while that species was minor (<9.7%) when CIP was supplied at 3.33 μg/L. There was, however, considerable variability in which species became dominant at the high CIP dose, depending on the concentrations of the other two antibiotics. For example, the species represented by the 757 bp fragment was predominant when the other two antibiotics also

were supplied at the high dose. It is not surprising to see more pronounced structure shifts associated with CIP exposure (than ERY or SMX) given that it is a broad spectrum antibiotic with a higher potency (*i.e.*, lower MIC) than the other two. The shift in community structure might be explained by the selection of CIP resistant bacteria (*e.g.*, 757 bp fragment), but we did not attempt to isolate and study the antibiotic resistance of individual strains.

Shifts in bacterial community composition in response to antibiotics have been reported previously, but typically for single antibiotics at much higher concentrations than those used in this work. For example, shifts in microbial composition were induced in soil bacteria exposed to lincomycin (>150 μg/L) (Čermák *et al*., 2008) and also for marine sediment bacteria exposed to CIP (>200 μg/L) (Naslund *et al*., 2008). Similarly, Knapp *et al*. (2008) reported increases in the presence (at 250 μg/L) and selection rate (at 20 μg/L) of antibiotic resistance genes in simulated surface waters exposed to oxytetracycline at concentrations of 250 μg/L and 20 μg/L, respectively. In contrast, Muñoz-Aguayo *et al*. (2007) concluded that antibiotic resistance (in terms of resistant gene diversity) was significant (*i.e.*, higher than the control) for simulated river ecosystems exposed to chlortetracycline at 800 μg/L, but not at 8 μg/L. The research results described herein, therefore, involve shifts at lower concentrations of antibiotics than has been previously observed, although this is likely caused by the relatively high potency of ciprofloxacin at low concentrations (*i.e.*, the "clinical definition" of resistance to ciprofloxacin is growth at concentrations > 200 μg/L).

The bacterial community also responded to the high CIP concentration by producing or retaining more biomass, which resulted in lower k_{VS} values for those runs. Biomass or VS in the reactor is comprised of active bacteria, inactive bacteria, and EPS. The elevated VS may be due to an increase in the amount of inactive cells or protective/sacrificial phenotypes that can accumulate in biofilms exposed to toxic compounds (Hoyle and Costerton, 1991). The high VS may also be due to the increased production of extracellular polymeric substances (EPS) as a defense mechanism to sorb antibiotics and reduce the aqueous concentration (Chapter 3). Dosed alone at sub-inhibitory concentrations, tetracycline, and quinupristin-dalfopristin, and ERY increased *Staphylococcus epidermidis* biofilm formation 9-fold, 11-fold, and 2.5-fold, respectively (Rachid *et al*., 2000). Given the relatively small mass fraction of bacteria in biofilm (5-10%; Bryers, 2000) and the inability to differentiate active biomass from inactive biomass and EPS with VS measurements, other direct measures of active biomass would be better for normalizing substrate degradation rates. Unfortunately, such approaches (*e.g.*, live-dead staining with microscopy or flow cytometry) were not used given the time and effort involved and the potential difficulties in interpreting the results from such techniques when applied to microbial communities comprised of a wide variety of bacteria with a range of physiological states (Berney *et al*. 2007, Pascaud *et al*. 2009).

The control condition k_{VS} values (1.64 ± 0.23 and 1.72 ± 0.12 mg C/mg VS/day) determined in this research fall within the range of values reported by others. For example, Rittmann *et al*. (1986) supplied filter columns with acetate-amended oligotrophic lake water (13.8 °C, pH 7) and reported a k_{VS} value of 0.47 mg C/mg VS/day

for the multi-species biofilm community. Also, Martin (1990) reported a k_{VS} of 7.17 mg C/mg VS/day for acetate-fed *Pseudomonas aeruginosa* biofilm growing in filter columns (21-22.5 °C, pH 7.94-8.14). Conversely, the control run K_s values obtained in this work (4 ± 1 and 0.4 ± 0.4 mg C/L) fall outside of the range of values reported by Rittmann *et al*. (1986) and Martin (1990) (5.27 mg C/L and 41 mg C/L, respectively). Differences in kinetic parameters can be attributed to differences in the bacterial species and conditions (*e.g.*, temperature) used in the experiments. In particular, K_s values for the same substrate tend to vary widely (*e.g.*, Zhang *et al*., 2009). Also, there is a large amount of uncertainty in our K_s values that results from the few data points in the low concentration range in the kinetic experiments (Figure 2.2).

As mentioned previously, the CFRAB was selected as a model system for this work while the application is packed-bed biofiltration systems. A relatively simple analysis was performed to compare the CFRAB to various biofiltration systems (Table 2.3). Using the approach described by Gagnon and Huck (2001), the external mass transfer rate (k_m) of the CFRAB for our experimental conditions is 1.46×10^{-5} m/s. The k_m values for biofiltration systems were computed using the approach described by Grigorescu and Hozalski (2010). The CFRAB k_m value is similar to that of slow sand filtration (1.71×10^{-5} m/s) but the HRT is about an order of magnitude less. Damköhler numbers ($Da = k_{areal}/k_m \cdot S_{eff}$) were computed to determine the relative importance of mass transfer and biodegradation rate in our system (Bailey and Ollis, 1986). Mass transfer limited conditions are indicated by a $Da > 1$, and reaction limitations are indicated by a $Da < 1$. Computed Da values for our system ranged from 0.19 to 6.36 (k_{areal} from 1.2 to 1.6 mg

C/cm^2/day, and S_{eff} from 0.2 to 5 mg C/L) during the kinetics experiments, suggesting that at the high substrate doses the system was biodegradation rate limited. Overall, the reasonable agreement between our control run kinetic parameters and those reported in the literature together with the results in Table 2.3 suggest that the CFRAB was a suitable model system for our work.

Table 2.3. Comparison of external mass transfer rates (k_m) and hydraulic residence times (HRT) for the CFRAB used in this research with biofiltration systems used for water treatment.

System	k_m (m/s)	HRT[A]
CFRAB[B, F]	1.46 x 10^{-5}	0.5 hours
Rapid gravity filtration[C, G]	3.54 x 10^{-5}	0.01 to 0.08 hours
Slow sand filtration[D, G]	1.71 x 10^{-5}	2 to 6 hours[H]
Bank filtration[E, G]	0.79 x 10^{-5}	5 to 100 days[I]

[A]Hydraulic residence time
[B] d=0.15 m; u=0.387 m/s
[C] d_p=1 mm; v=10 m/hr; ε=0.4; HRT (with 1-meter bed length) of 0.04 hours.
[D] d_p=0.3 mm; v=0.1 m/hr; ε=0.4; HRT (with 1-meter bed length) of 4 hours.
[E] d_p=0.3 mm; v=0.01 m/hr; ε=0.4; HRT (with 10-meter bed length) of 16.7 days.
Water properties: ρ_w=1000kg/m^3; μ_w=0.001 kg/m·sec
Diffusivity of acetate in water, D_w=1.09 x 10^{-9} m^2/sec
[F] $k_m=ShD_w/d$; Sherwood No., $Sh=0.023Re^{0.83}Sc^{0.33}$; Reynolds No., $Re=du\rho_w/\mu_w$; Schmidt No., $Sc=\mu_w/D_w\rho_w$
[G] $k_m=ShD_w/d_p$; $Sh=1.09\varepsilon^{-2/3}Re^{0.33}Sc^{0.33}$; $Re=d_pv\rho_w/(1-\varepsilon)\mu_w$; $Sc=\mu_w/D_w\rho_w$
[H]range of values estimated from information provided by Kawamura (1991) assuming a porosity of 0.4
[I]from Kuehn and Mueller (2000)

Given that antibiotics typically occur at sub-µg/L concentrations in surface waters, it is unlikely that antibiotics will affect biofiltration performance. Our results suggest, however, that some effects are possible if concentrations of low MIC antibiotics, such as CIP, occur at low µg/L concentrations. The potential effects include shifts in microbial community composition and increased production or accumulation of biomass. Although acetate degradation rates were not affected in our experiments, shifts in microbial community structure might negatively impact biodegradation of specific compounds if those compounds are only degraded by a single or few species and those species are

sensitive to the antibiotics. Certainly more work is needed to investigate the affects of other antibiotics and combinations not tested here on not only aerobic heterotrophs but also other bacteria of relevance in biofiltration systems such as ammonia oxidizers. Furthermore, increases in biomass accumulation could be problematic if such increases lead to more rapid headloss development in the biofilters. In addition to possible effects on biofilter performance, another potential concern is that the occurrence of low levels of antibiotics in surface waters could increase the proliferation of antibiotic resistant bacteria in biofilters.

2.5 Conclusions

The results of this research indicated that mixtures of antibiotics at the sub-μg/L concentrations typically observed in surface waters are unlikely to affect the overall performance of biofiltration systems. Should concentrations of low MIC antibiotics such as CIP enter the low μg/L concentration range, there is some concern that negative effects might occur, such as shifts in bacterial community structure and increased accumulation of biomass. Thus, diligence in the controlled use and disposal of antibiotics is warranted to prevent antibiotic concentrations in aquatic systems from increasing further.

Acknowledgements

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2.6 References

Acar, J.F. (2000) Antibiotic synergy and antagonism. Medical Clinics of North America 84(6), 1391-1406.

Alexy, R., Kümpel, T., Kümmerer, K. (2004) Assessment of degradation of 18 antibiotics in the Closed Bottle Test. Chemosphere 57**(**6), 505-512.

Ash, R.J., Mauck, B., Morgan, M. (2002) Antibiotic resistance of gram negative bacteria in rivers, United States. Emerging Infectious Diseases 8(7), 713-716.

Bailey J. E., Ollis D. F. (1986) Biochemical Engineering Fundamentals. McGraw-Hill, Inc., New York, NY.

Batt, A.L., Bruce, I.B., Aga, D.S. (2006) Evaluating the vulnerability of surface waters to antibiotic contamination from varying wastewater treatment plant discharges. Environmental Pollution142(2), 295-302

Benotti, M.J., Trenholm, R.A., Vanderford, B.J., Holady, J.C., Stafford, B.D., Snyder, S.A. (2009) Pharmaceuticals and endocrine disrupting compounds in US drinking water. Environmental Science &Technology 43(9), 597-603.

Berney,M., Hammes, F., Bosshard, F., Weilenmann, H.U., Egli, T. (2007) Assessment and interpretation of bacterial viability by using the LIVE/DEAD *Bac*Light Kit in combination with flow cytometry. Applied Environmental Microbiology **73,** 3283–3290.

Boxall, A.B.A., Kolpin, D.W., Halling-Sørensen, B., Tolls, J. (2003) Are veterinary medicines causing environmental risks? Environmental Science and Technology 37(15), 286A-294A.

Bryers, J. (Ed.) (2000) *Biofilms II-Process Analysis and Applications*. Wiley, New York, NY.

Cardinale, M., Brusetti, L., Quatrinia, P., Borin, S., Puglia, A., Rizzi, A., Zanardini, E., Sorlini, C., Corselli, C., Daffonchio, D. (2004) Comparison of different primer sets for use in automated ribosomal intergenic spacer analysis of complex bacterial communities. Applied and Environmental Microbiology 70(10), 6147-6156.

Čermák, L., Kopecký, J., Novotná J., Omelka, M., Parkhomenko, N., Plháčková, K., Ságová-Marečková, M. (2008) Bacterial communities of two contrasting soils reacted differently to lincomycin treatment. Applied Soil Ecology 40, 348–358.

Černohorská, L., Voltava, M. (2008) Antibiotic Synergy against biofilm-forming *Pseudomonas aeruginosa*. Folia Microbiology 53(1), 57–60.

Collins, M.R., Eighmy, T.T., Fenstermacher, J.M., Spanos, S.K (1989) Modifications to the Slow Sand Filtration Process For Improved Removals of Trihalomethane Precursors. AWWARF, Denver, CO.

Costanzo, S.D., Murby, J., Bates, J. (2005) Ecosystem response to antibiotics entering the aquatic environment. Marine Pollution Journal 51(1-4), 218-223.

Cottarel, G., Wierzbowski, J. (2007) Combination drugs, an emerging option for antibacterial therapy. Trends in Biotechnology 25(12), 547-555.

Drakapoulos, A.I., Ioannou, P.C. (1997) Spectrofluorimetric study of the acid-base equilibria and complexation behavior of the fluoroquinolone antibiotics ofloxacin, norfloxacin, ciprofloxacin and pefloxacin in aqueous solution. Analytical Chemistry Acta 354(1), 197-204.

Gagnon, G.A., Huck, P.M. (2001) Removal of easily biodegradable organic compounds by drinking water biofilms: analysis of kinetics and mass transfer. Water Research 35(10):2554-2564.

Ghosh, G.C., Okuda, T., Yamashita, N. (2009) Occurrence and elimination of antibiotics at four sewage treatment plants in Japan and their effects on bacterial ammonia oxidation. Water Science and Technology 59(4), 779-786.

Grigorescu, A.S., Hozalski, R.M. (2010) Modeling HAA biodegradation in biofilters and water distribution systems. Journal AWWA 102(7), 67-80.

Hansch, C., Leo, A., Hoekman, D. (1995) Exploring QSAR: Volume 2: Hydrophobic, Electronic, and Steric Constants. American Chemical Society, Washington, D.C.

Heberer, T., Massmann, G., Fanck, B., Taute, T., Dünnbier, U. (2008) Behaviour and redox sensitivity of antimicrobial residues during bank filtration. Chemosphere 73(4), 451-460.

Heberer, T., Mechlinski, A., Fanck, B., Knappe, A., Massmann, G., Pekdeger, A., Fritz, B. (2004) Field studies on the fate and transport of pharmaceutical residues in bank filtration. Ground water Monitoring & Remediation 24(2), 70-77.

Hiscock, K.M., Grischek, T. (2002) Attenuation of groundwater pollution by bank filtration. Journal of Hydrology 266, 139-144.

Hoyle, B.D., Costerton, J.W. (1991) Bacterial resistance to antibiotics: the role of biofilms. Progress in Drug Research 37, 91–105.

Kawamura, S. (1991) *Integrated Design of Water Treatment Facilities*. John Wiley and Sons, New York.

Kennedy A.F., Sutherland I.W. (1987) Analysis of bacterial exopolysaccharides. Biotechnology and Applied Biochemistry 9(1), 12–19.

Knapp, C.W., Engemann, C.A., Hanson, M.L., Keen, P.L., Hall, K.J., Graham, D.W. (2008) Indirect evidence of transposon-mediated selection of antibiotic resistance genes in aquatic systems at low-level oxytetracycline exposures. Environmental. Science and Technology 42, 5348–5353.

Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B.; Buxton, H.T. (2002) Pharmaceuticals, hormones, and other organic wastewater constituents in US streams, 1999-2000: a national reconnaissance. Environmental Science and Technology 36(6), 1202-1211.

Kuehn, W., Mueller, U. (2000) Riverbank filtration: an overview. Journal AWWA 92(12), 60-69.

Kuo, C-Y. (1998) Improved application of ion chromatographic determination of carboxylic acids in ozonated drinking water. Journal of Chromatography A 804, 265–272.

Lin, A.Y.-C., Yu, T.-H., Lateef, S.K. (2009) Removal of pharmaceuticals in secondary wastewater treatment processes in Taiwan. Journal of Hazardous Materials 167(1-3), 1163-1169.

Lorian, V., Ed. (1996) Antibiotics in Laboratory Medicine, 4th ed. Lippincott Williams & Wilkins, Baltimore, MD.

Martin, R.E. (1990) Quantitative description of bacterial deposition and initial biofilm development in porous media. Ph.D. Dissertation. The Johns Hopkins University, Baltimore, MD.

Mellon, M., Benbrook, C., Benbrook, K.L. (2001) Hogging it: estimation of antimicrobial use in Livestock. Union of Concerned Scientists: Cambridge, MA.

Monzón, M.; Oteiza, C.; Leiva, J, Amorena, B. (2001) Synergy of different antibiotic combinations in biofilms of *Staphylococcus epidermidis*. Journal of Antimicrobial Chemotherapy 48, 793-801.

Muñoz-Aguayo, J., Lang, K.S., LaPara, T.M., González, G., Singer, R.S. (2007) Evaluating the effects of chlortetracycline on the proliferation of antibiotic-resistant bacteria in a simulated river water ecosystem. Applied Environmental Microbiology 73(17), 5421-5425.

O'Shea, R., Moser, H.E. (2008) Physicochemical properties of antibacterial compounds: implications for drug discovery. Journal of Medicinal Chemistry 51(10), 2871-2878.

Naeem, S., Li, S. (1997) Biodiversity enhances ecosystem reliability. Nature 390, 507-509.

Naslund, J., Hedman, J.E., Agestrand, C. (2008) Effects of the antibiotic ciprofloxacin on the bacterial community structure and degradation of pyrene in marine sediment. Aquatic Toxicology 90, 223–227.

Nelson, D.K., LaPara, T.M.. Novak, P.J. (2010) Effects of ethanol-based fuel contamination: microbial community changes, production of regulated compounds, and methane generation. Environmental Science and Technology 44(12), 4525-4530.

Neu, H.C. (1991) Synergy and antagonism of combinations with quinolones. European Journal of Microbiology and Infectious Diseases 10(4), 255-261.

Pascaud, A., Amellal, S., Soulas M.L., Soulas, G. (2009) A fluorescence-based assay for measuring the viable cell concentration of mixed microbial communities in soil. Journal of Microbiological Methods 76(1), 81-87.

Pena, A., Chmielova, D., Lino, C.M., Solich, P. (2007) Determination of fluorquinolone antibiotics in surface waters from Mondego River by high performance liquid chromatography using amonolithic column. Journal of Separation Science 30, 2924-2928.

Rachid, S., Ohlsen, K., Witte, W., Hacker, J. (2000) Effect of subinhibitory antibiotic concentrations on polysaccharide intercellular adhesin expression in biofilm-forming *Staphylococcus epidermidis*. Antimicrobial Agents and Chemotherapy 44(12), 3357–3363.

Renew, J.E., Huang, C.H. (2004) Simultaneous determination of fluoroquinolone, sulfonamide, and trimethoprim antibiotics in wastewater using tandem solid phase extraction and liquid chromatography-electrospray mass spectrometry. Journal of Chromatography A 1042(1-2), 113-121 .

Rittmann, B.E., Crawford, L., Tuck, C.K., Narnkung, E. (1986) In situ determination of kinetic parameters for biofilms: isolation and characterization of oligotrophic biofilms Biotechnology and Bioengineering 28, 1753-1760.

Rooklidge, S.J., Miner, JR., Kassim, T.A., Nelson, P.O. (2005) Antimicrobial contaminant removal by multistage slow sand filtration. Journal AWWA 97(12), 92-100.

Snyder, S.A., Adham, S., Redding, A.M., Cannon, F.S., DeCarolis, J., Oppenheimer, J., Yoon, Y. (2007) Role of membranes and activated carbon in the removal of endocrine disruptors and pharmaceuticals. Desalination 202, 156-181.

Thiele-Bruhn, S., Beck, C. (2005) Effects of sulfonamide and tetracycline antibiotics on soil microbial activity and microbial biomass. Chemosphere 59, 457-465.

von Canstein, H.V., Kelly, S., Li, Y., Wagner-Dobler, I. (2002) Species diversity improves the efficiency of mercury-reducing biofilms under changing environmental conditions. Applied Environmental Microbiology 68(6), 2829-2837.

Weber-Shirk, M., Dick, R.I. (1997) Physical-chemical mechanisms in slow sand filters. Journal AWWA, 89(1), 87-100.

Yang, Li-.H., Ying, G-.G., Su, H,C., Stauber, J.L., Adams, M.S., Binet, M.T. (2008) Growth-inhibiting effects of 12 antibacterial agents and their mixtures on the freshwater microalga *Pseudokirchneriella subcapitata*. Environmental Toxicology and Chemistry 27(5), 1201–1208.

Zhang, P., LaPara, T.M., Goslan, E., Xie, Y., Parsons, S., Hozalski, R.M. (2009) Biodegradation of haloacetic acids by bacterial isolates and enrichment cultures from drinking water systems. Environmental Science and Technology, 43(9), 3169–3175.

Chapter 3. Sorption of Antibiotics to Biofilm[†]

Using a continuous-flow rotating annular bioreactor, sorption of three selected antibiotics (sulfamethoxazole (SMX), ciprofloxacin (CIP), and erythromycin (ERY)) to bacterial biofilm was investigated. CIP had the greatest biofilm partition coefficient (K_{oc} = 92,000 ± 10,000 L/kg) followed by ERY (K_{oc} = 6,000 ± 1,000 L/kg) and then SMX (K_{oc}= 4,000 ± 1,000 L/kg). Antibiotic sorption to biofilm did not correlate with experimentally-determined K_{ow} values (CIP: -0.4; ERY: 0.98; SMX: <-0.59 at pH 7), suggesting that hydrophobic interactions do not drive the sorption of these relatively hydrophilic compounds to biofilm. It appears that speciation (*i.e*. charge) and molecular size of the antibiotics are important in explaining their sorption to typically negatively charged biofilm. SMX is neutral to negatively charged at circumneutral pH while CIP and ERY are both positively charged. The decreased extent of sorption of ERY relative to CIP is likely due to the larger molecular size of ERY that results in a decreased rate of mass transfer (*i.e*. diffusion) to and through the biofilm. In conclusion, the results of this research suggest that hydrophobic interactions (predicted by K_{ow}) do not control sorption of relatively hydrophilic antibiotics to biofilm and that antibiotic speciation and molecular size are important factors affecting the interactions between antibiotics and biofilm.

[†]Reproduced with permission in part from "Wunder, D.B., Bosscher, V.A., Cok, R.C., Hozalski, R.M. (2010) Sorption of antibiotics to biofilm. Water Research, *in press*: doi:10.1016/j.watres.2010.11.013"

3.1 Introduction

Antibiotics have been detected in surface waters around the world at concentrations up to 1.9 μg/L (Kolpin *et al*., 2002; Pena *et al*., 2007). The presence of antibiotics in surface waters is of concern for several reasons. First, antibiotic resistance can develop in bacteria with exposure to sub-inhibitory concentrations (Ash *et al.*, 2002). Also, aquatic organisms (algae, nitrifying bacteria, zooplankton) can be adversely affected by mixtures of antibiotics at low concentrations (*i.e*., 0.1 to 50 μg/L) (Flaherty and Dodson, 2005; Yang *et al*., 2008; Ghosh *et al*., 2009).Finally, although the human health effects of sustained exposure to antibiotics at sub-therapeutic doses are currently unknown, there is heightened public awareness over the presence of antibiotics and other pharmaceutical compounds in drinking water supplies (Benotti *et al*., 2009). Thus, approaches to remove antibiotics and other pharmaceutical compounds from water supplies are a priority.

Antibiotics are not effectively removed via conventional water treatment (*i.e*., coagulation/flocculation/ sedimentation/filtration) or lime softening ($\leq$33%, Adams *et al*., 2002; Westerhoff *et al*., 2005). Free chlorine (1 mg/L for 40 minutes) effectively removes ($\geq$90%) some antibiotics (sulfonamides, carbodox, and trimethoprim) from surface water, although SMX may reform during dechlorination (Adams *et al*., 2002; Dodd and Huang, 2004). Unfortunately, little is known about the products of antibiotic chlorination and their activity and toxicity. Chlorine dioxide and ozone also effectively remove antibiotics (*e.g.*, sulfonamides and macrolides) at reasonable doses and contact times (Huber *et al*., 2005; Westerhoff *et al*., 2005), but this is not the case for chloramines (Chamberlain and Adams, 2006). Fresh granular activated carbon (GAC) effectively removes SMX and

ERY (7.6-minute empty-bed contact times), with spent GAC still exhibiting some removal (<40% for SMX and <55% for ERY) (Westerhoff *et al*., 2005; Snyder *et al*., 2007). Regarding membrane filtration, only reverse osmosis and nanofiltration are effective at rejecting antibiotics (Nghiem *et al*., 2005; Snyder *et al*., 2007). Finally, photodegradation of tetracyclines, quinolones and ionized sulfonamides occurs in low turbidity waters (Tourniainen *et al.*, 1997; Moore and Zhou, 1994) and may contribute to observed removals in treatment facilities using UV disinfection systems.

Biofiltration systems, including slow-rate filtration (*i.e*., slow sand filtration, bank filtration) and biologically-active rapid filtration, have been used in the water industry for many decades, especially in Europe (Bouwer and Crowe, 1988; Hiscock and Grischek, 2002). Interest in biofiltration in the U.S. has increased in recent years because of the many potential water quality benefits these systems provide. For example, biofilters effectively remove a variety of organic pollutants including: disinfection byproduct precursors, pesticides, and pharmaceuticals (Eighmy *et al.*, 1993, Collins *et al.*, 1999, Hiscock and Grischek, 2002; Weiss *et al*., 2003). Removal mechanisms include biodegradation and sorption, with potential sorbents including the filter media (*e.g.*, GAC), natural organic matter (NOM) sorbed onto the filter media, and biofilm. Although there are reports of antibiotic sorption to GAC (Westerhoff *et al*., 2005; Snyder *et al*., 2007), sand (Thiele-Bruhn, 2003), manure, and digested sludge (Loke *et al*., 2002; Carballa *et al*., 2008), we are unaware of any studies concerning antibiotic sorption to biofilm. Understanding antibiotic sorption to biofilm could be useful for predicting the fate of antibiotics in biofiltration systems used for treatment of water or wastewater.

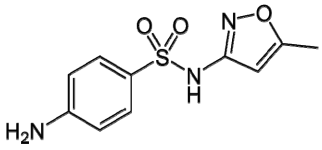
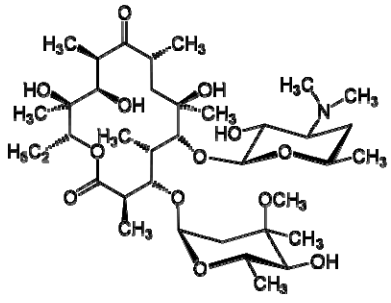
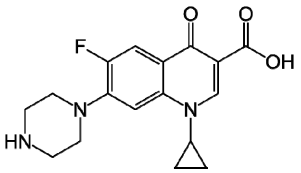
Herein, we report on the results of laboratory experiments performed to quantify the sorption of three selected antibiotics to biofilm as a first step in characterizing the fate of antibiotics in biofilters.

3.2 Materials and Methods

3.2.1 Antibiotics, Chemicals, and Reagents

The sorption of three selected antibiotics (Table 3.1) to biofilm was investigated using a continuous-flow rotating annular bioreactor (CFRAB). Sulfamethoxazole (SMX), Erythromycin (ERY), and Ciprofloxacin (CIP) were selected for the following reasons: (1) they represent three prominent classes of antibiotics with differing chemical characteristics and (2) they have been detected in surface water.

Table 3.1. Antibiotics Selected for Study

Sulfamethoxazole	Erythromycin	Ciprofloxacin
$C_{10}H_{11}N_3O_3S$	$C_{37}H_{67}NO_{13}$	$C_{17}H_{18}FN_3O_3$
MW = 253.3	MW = 733.9	MW = 331.3
pK_{A1} = 1.85, pK_{A2} = 5.60	pK_A = 8.90	pK_{A1} = 3.0, pK_{A2} = 6.1, pK_{A3} = 8.7, pK_{A4} = 10.6
		
$SMX^+ \leftrightarrow SMX \leftrightarrow SMX^-$	$ERY^+ \leftrightarrow ERY$	$CIP^{3+} \leftrightarrow CIP^{2+} \leftrightarrow CIP^+ \leftrightarrow CIP \leftrightarrow CIP^-$
pK_{A1} pK_{A2}	pK_A	pK_{A1} pK_{A2} pK_{A3} pK_{A4}

pK_A values from Qiang and Adams (2004)

SMX (a sulfonamide) and ERY (a macrolide) occur in surface waters at concentrations up to 1.9 and 1.7 μg/L, respectively (Kolpin *et al*., 2002). CIP is a fluoroquinolone with reported surface water concentrations of up to 119 ng/L (Pena *et al*., 2007). The *octanol-water partition coefficient* (K_{ow}), defined as the concentration of a chemical in octanol to that in water at equilibrium, is a commonly used parameter for predicting the fate of a chemical in the environment or of a pharmaceutical compound in the human body. Compounds with relatively high K_{ow} are more likely to partition to natural organic matter, bioaccumulate in aquatic organisms, or partition into hydrophobic compartments in the human body such as lipid bilayers. Reported logK_{ow} (pH 7.4 and 25 °C) values for SMX, ERY, and CIP are -0.9, 1.58, and -1.1, respectively (Hansch *et al*., 1995; Drakapoulos *et al*., 1997). The logK_{ow} determined at pH 7.4 (*i.e*., the physiological pH of blood serum) is often termed the *octanol-water distribution coefficient* (log$D_{7.4}$). For ionizable compounds, the logK_{ow} value determined at a pH where the neutral chemical species predominates is also called the *octanol-water partition coefficient* but often expressed as log*P*. Reported log*P* values for SMX, ERY, and CIP are 0.89, 3.06, and 0.4, respectively (Drakapoulos *et al*., 1997; McFarland *et al*., 1997; Congliang *et al.,* 2007). The pH values where these compounds are predominantly neutral (*i.e*. >95% of species; 4.3 for SMX, 10.2 for ERY, and ~10.0 for CIP), however, are obviously quite different from the circumneutral pH values typical of most natural waters.

SMX (≥98% purity), ERY (≥95% purity), CIP (≥98% purity), and octanol (>99% purity, reagent grade) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sulfamerazine (SMR; ≥99% purity; Sigma-Aldrich), Oleandomycin phosphate (OLE;

≥96% purity; MP Biomedicals, Solon, OH, USA), and Clinafloxacin hydrochloride (CLN; ≥98% purity; LKT Laboratories, Saint Paul, MN, USA) served as internal standards. The internal standards were selected based on their structural similarity to the respective antibiotics of interest and their use in comparable work (Zabniski *et al*., 1995; McArdell *et al*., 2003; Scribner *et al.*, 2003, Gobel *et al*., 2004; Renew and Huang 2004). All antibiotic compounds were used as received. Suwannee River NOM (SRNOM) was purchased from the International Humic Substances Society (Saint Paul, MN, USA).

3.2.2 Experiments

3.2.2.1 Sorption Experiments

Sorption experiments were conducted using a continuous-flow rotating annular bioreactor (CFRAB) system (Figure 3.1; Model 1120 LS, BioSurface Technologies Bozeman, MT) following the method of Headley *et al*. (1998). Sometimes referred to as rototorque reactors, CFRABs have been used for biofilm research for about two decades including investigations of biofilm heterogeneity (Gjaltema *et al.*, 1994), organic contaminant sorption to biofilm (Headley *et al.*, 1998), and the kinetics of organic compound biodegradation by biofilms (Gagnon and Huck, 2001). The CFRABs used in this work consisted of a rotating inner cylinder (140 mm diameter) with 20 flush-mounted polycarbonate plastic slides inside of a stationary glass outer cylinder with an inner diameter of 155 mm. The total open or liquid volume was 1 L. The advantages of the CFRAB system for biofilm studies are the high surface area to volume ratio, removable slides for biofilm examination and quantification, and the ability to control the wall shear

stress, determined by the rotational speed of the inner cylinder, independently of the hydraulic residence time.

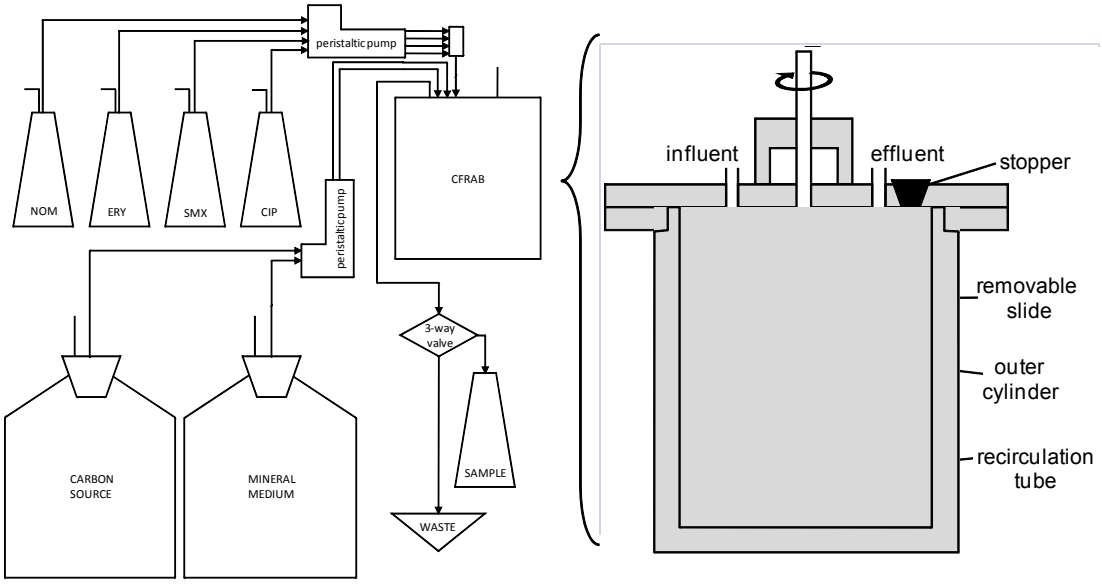


Figure 3.1. CFRAB Apparatus.

The CFRAB was autoclaved prior to use. Trickling filter rock media from the Clean Water Plant in Wyoming, MI was used for seeding the reactor in order to establish a biofilm. This biofilm source was chosen because it provided a diverse community for colonization of the reactor and was proximate to the laboratory. For seeding, mineral medium and acetate (100 mg C/L) were continuously pumped through a 5-liter polyplopylene reservoir (also used for collection) containing five biofilm-coated tricking filter rocks upstream of the CFRAB. The mineral medium (pH 7.25 ± 0.3) contained (per liter of water): 43.8 mg/L K_2HPO_4, 17 mg/L KH_2PO_4, 62.4 mg/L Na_2HPO_4, 45.0 mg/L $MgSO_4$, 0.5 mg/L $FeCl_3$-$6H_2O$, 5.4 mg/L NH_4Cl, and 55 mg/L $CaCl_2$. The upstream reservoir was taken off-line after a day of seeding. The CFRAB was operated with

continuous feeding of mineral medium and 2 mg C/L acetate for 20-days to reach steady-state conditions (defined as < 20% variation in consecutive effluent plate counts) before initiating the sorption experiments. The CFRABs were operated with a 30-minute residence time, 100 rpm mixing, and at a temperature of 25°C. To minimize the potential for antibiotic photodegradation, all CFRAB apparatus components were covered, amber bottles were used for sample collection, and the laboratory was unlighted except for low background light during maintenance and sampling.

Sorption experiments were conducted by initiating a step input of either a single antibiotic or a mixture of the three antibiotics and measuring the effluent concentration over time for up to 25 hours. The influent antibiotic concentrations applied in our experiments were 0.33 μg/L (*i.e.* low concentration) and 3.33 μg/L (*i.e.* high concentration). Antibiotic and acetate feed solutions were prepared daily by diluting concentrated stock solutions. At the initiation of an experimental run, the reactor was spiked to the target antibiotic feed concentration (either 0.33 or 3.33 μg/L of antibiotic) using an autopipette and the antibiotic feed was turned on. One-liter samples were collected every 30 minutes for the first five to six hours, and then with decreasing frequency through 25 hours. For most runs effluent samples were collected after ceasing antibiotic feed; effluent concentrations decreased to below detection limits after 24 hours. The CFRAB was operated without antibiotic feed for at least 5 days between runs and effluent samples were collected before the next run to ensure that no residual antibiotics remained from the previous run. The effect of NOM on antibiotic sorption to biofilm was investigated by pre-equilibrating the CFRAB with NOM (1 mg/L as C) for 24 hours prior

to initiating the feed of antibiotic and continuing to feed NOM throughout the experimental run.

Upon completing the last sorption experiment, the reactor biofilm was collected for determination of total organic carbon (TOC). CFRAB biofilm was detached from the removable slides via scraping and washing with distilled water and transferred to a glass flask. The biofilm was liquefied via acidification to pH 2 with phosphoric acid prior to TOC analysis (APHA, 1995).

3.2.2.2 Octanol-Water Partitioning Coefficients

A modified shake flask method (USEPA, 1996) was used to determine the octanol-water partition coefficient (K_{ow}) of antibiotics as a function of pH (6, 7, and 8), calcium hardness (65 and 185 mg $CaCO_3$/L), and NOM (0 and 0.5 mg/L as C). The water phase consisted of phosphate-buffered mineral medium and the pH was adjusted by titration with either HCl or NaOH. For each experiment 14 mL of water was spiked with 1 mg/L of each antibiotic into a 17 mL polypropylene centrifuge tube and then shaken at 240 rpm on an orbital shaker for 12 hours to allow for equilibration prior to the addition of octanol. Then either 1.5 or 2.0 mL of octanol was added and the tube was shaken at 240 rpm on an orbital shaker for 12 hours to allow for equilibration. Each sample was then centrifuged at 3000 rpm for 100 minutes to separate the water and octanol phases and then 1 mL of octanol was transferred to an amber vial which was stored at < 4°C in the dark until analysis.

3.2.3 Analytical Methods

Immediately after collection from the CFRAB, the one-liter aqueous samples were filtered (0.45 μm glass fiber filter, Millipore, Billerica, MA, USA) to remove biofilm fragments and bacteria and then stored for up to 2 days at ~4°C prior to subsequent processing. The filtered samples were pre-concentrated via solid-phase extraction using 200 mg Oasis hydrophilic-lipophilic-balanced water-wettable copolymer cartridges (Waters, Milford, MA, USA). The methanol extracts (6 ml) were reduced to 1 mL via evaporation using nitrogen gas prior to analysis. Octanol samples were analyzed directly without any processing.

Liquid chromatography-mass spectrometry (LC-MS) (Agilent 1100 series) with positive ion electrospray detection in selective ion monitoring mode was used for analysis of antibiotics in water and in octanol. Retention times and *m/z* for the studied antibiotics and internal standards were: SMX (6.9 min and 254); SMR (6.4 min and 265); ERY (3.9 min and 716.5); ERY•H_2O (4.2 min and 734); OLE (3.8 min and 688.4); CIP (3.8 min and 332); and CLN (3.9 min and 366). The two sets of values for erythromycin reflect the anhydrous (ERY) and hydrated (ERY•H_2O) forms, the concentrations of which were summed to get the total erythromycin concentration. A Luna 5 micron C18(2) 100 angstrom 150 x 3 mm LC column (Phenomenex, Torrence, CA, USA) was used for separation. The LC-MS mobile phase consisted of 40% of 0.3% (v/v) formic acid in acetonitrile and 60% of 0.3% (v/v) formic acid in HPLC-grade water. An internal standard calibration method was used for antibiotic quantification in aqueous samples (Lindberg *et al*., 2004; Radjenovića *et al*., 2007), with SMR, CLN, and OLE serving as

internal standards in water samples for SMX, CIP, and ERY, respectively. Each sample was analyzed in duplicate and the results were averaged. Relative standard deviations (RSD) for the internal standards were between: 11 and 21% for OLE; 10 and 22% for SMR; and 13 and 22% for CLN. Method detection limits (MDL) were 0.05 μg/L for CIP and 0.1 μg/L for both SMX and ERY as determined according the approach described in Standard Methods (APHA, 1995). A similar LC-MS approach was used for octanol samples, but without internal standards. For octanol samples, 5-point calibration ($R^2 >$ 0.99) of antibiotic samples in octanol was used for quantification, with an MDL of 0.25 mg/L for each antibiotic.

3.2.4 Data Analysis

3.2.4.1 Sorption Experiments

For each sorption experiment the antibiotic concentration (C) versus time (t) data were fit to a non-steady state mass balance model assuming a well-mixed reactor and linear sorption (Headley *et al*., 1998)

$$\frac{dC}{dt} = \frac{Q(C_{feed} - C)}{K_{OC}M + V} \quad (1)$$

where: Q is the volumetric flow rate (L/min), C is the effluent antibiotic concentration (μg/L), C_{feed} is the influent antibiotic concentration (μg/L), K_{oc} is the biofilm organic carbon partition coefficient (L/kg), M is the mass of the biofilm organic carbon (kg), and V is the CFRAB volume (L). Integrating equation 1 yields

$$C = C_{feed} + \left[\frac{C_{feed} V}{K_{oc} M + V} - C_{feed} \right] e^{-kt} \tag{2}$$

where: k is the rate constant (1/min) and t is the time in minutes. The rate constant is a function of the partition coefficient K_{oc} (L/kg) as shown below

$$k = \frac{Q}{K_{oc} M + V} \tag{3}$$

TableCurve2D version 5.01 (Systat Software, Chicago, IL) was used to fit a simplified form of equation 1 ($C = C_{feed} + (a - C_{feed})e^{-kt}$) and solve for the two parameters, a and k. Because the magnitude of regressed values for the *a* term (*i.e.* $C_{feed}V/K_{oc}M + V$) were negligible compared to C_{feed} (*i.e.* a < 1 x 10^{-12} µg/L for runs with C_{feed} = 0.33 µg/L, and ranging from 1 x 10^{-12} to 0.43 µg/L for C_{feed} = 3.33 µg/L) in the initial fits, equation 2 was further simplified by setting *a* equal to zero. Hence, a final round of data fitting was done with a one-parameter model of the form ($C = C_{feed}(1 - e^{-kt})$) and then the K_{oc} values were determined from the regressed values for k and known values for *Q*, *M*, and *V*. It should be noted that no specific sorption mechanism is implied by the model and this study was not designed to elucidate underlying sorption mechanisms or the sorption sites involved. The assumption of linear sorption was believed to be reasonable because of the low antibiotic concentrations employed in the experiments. Biodegradation was not considered in the mass balance model because reported biodegradation rates for the studied antibiotics are slow (even after allowing for acclimation) compared to the duration of each experimental run (Al-Ahmad *et al*., 1999; Alexy *et al*., 2004; Drillia *et al*., 2005a).

3.2.4.2. Octanol-Water Partition Coefficient Experiments

Octanol-water partition coefficients (K_{ow}) were determined from a system mass balance. The antibiotic mass in octanol at equilibrium was computed from the measured antibiotic concentration and known volume of octanol. Knowing the total mass of antibiotic added, the antibiotic mass in water at equilibrium was determined by difference. The K_{ow} is simply the ratio of the concentration of antibiotic in octanol to that in water.

3.3 Results

3.3.1 Antibiotic Sorption Experiments

Representative plots of effluent antibiotic concentration (normalized to the feed concentration) versus time are provided in Figure 3.2 and the sorption parameters and correlation coefficient (R^2) values for all experimental runs are provided in Table 3.2. No loss of antibiotics was observed in control experiments run with a clean (*i.e*. no biofilm) reactor (Figure 3.2), indicating that biofilm was responsible for the observed losses in the other experiments.

The R^2 values ranged from 0.414 to 0.992 (with a mean of 0.858) and the quality of the fit increased with increasing extent of sorption as CIP exhibited the highest K_{oc} and R^2 values. Given the dynamic and complex nature of the biological sorbent (*i.e.* biofilm), the R^2 values were deemed to be acceptable. With the exception of ERY and SMX in the high concentration (3.33 μg/L) mixture runs, results were reproducible.

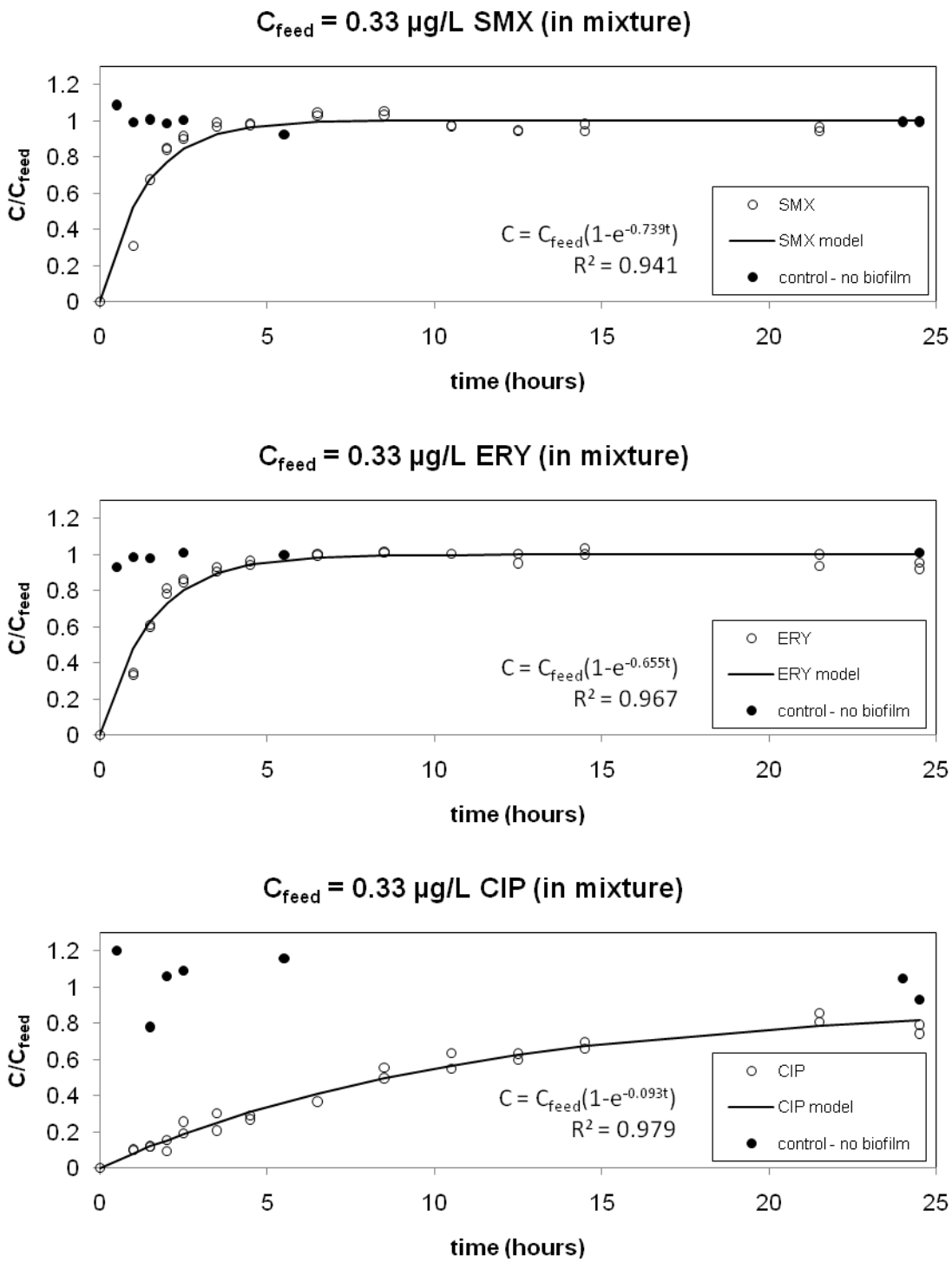


Figure 3.2. Representative plots of effluent antibiotic concentration (normalized to C_{feed}) versus time for SMX, ERY, and CIP, fed as a mixture (0.33 µg/L, each antibiotic). The symbols represent the experimentally determined values (open = with biofilm and closed = control) and the solid lines represent the model fits.

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Table 3.2. Regression Model Biosorption Parameters

Feed Condition	Concentration (µg/L)	k (hr^{-1})	K_{oc} (L/kg)	R^2	K_{oc} (L/kg)[A]
CIP (alone)	0.33	0.10	75,000	0.952	92,000 ±10,000
CIP (alone)	3.33	0.11	74,000	0.815	
CIP (mixture)	0.33	0.11; 0.09	71,000; 84,000	0.802; 0.979	
CIP (mixture)	3.33	0.06; 0.06	126,000; 124,000	0.976; 0.992	
ERY (alone)	0.33	0.95	5,000	0.706	6,000±1,000
ERY (alone)	3.33	0.74	7,000	0.925	
ERY (mixture)	0.33	0.69; 0.66	8,000; 8,000	0.703; 0.969	
ERY (mixture)	3.33	1.25; 0.68	2,000; 8,000	0.601; 0.836	
SMX (alone)	0.33	0.72	7,000	0.946	4,000±1,000
SMX (alone)	3.33	3.49	0[B]	0.883	
SMX (mixture)	0.33	0.97; 0.74	4,000; 7,000	0.414; 0.941	
SMX (mixture)	3.33	1.05; 2.41	4,000; 0[C]	0.988; 0.954	
SMX (mixture), 1 mg C/L NOM	3.33	1.28	2,000	0.975	
ERY (mixture), 1 mg C/L NOM		1.55	1,000	0.696	
CIP (mixture), 1 mg C/L NOM		0.10	74,000	0.966	

Note: two values reported for replicate runs

[A]Mean ± standard error

[B]Negative value for K_{oc} (-2000) obtained from Equation 2 was set to zero

[C]Negative value for K_{oc} (-1000) obtained from Equation 2 was set to zero

The sorption rate constant (k) values for CIP (0.06 to 0.11 hr^{-1}) were markedly slower than for ERY (0.66 to 1.25 hr^{-1}) and SMX (0.74 to 3.49 hr^{-1}). CIP consistently had the greatest K_{oc} values (74,000-126,000 L/kg), regardless of feed conditions. The K_{oc} values for ERY and SMX were similar and about an order of magnitude lower than that for CIP. In general, the K_{oc} values were unaffected by changes in feed concentration and were similar whether the compounds were fed alone or as a mixture of the three antibiotics. In limited testing, antibiotic sorption to biofilm did not appear to be affected by the presence of SRNOM. The K_{oc} values from this work are plotted versus K_{ow} in Figure 3.3 along with data from other studies in which sorption of organic compounds to biofilm was investigated.

3.3.2 Octanol-Water Partition Coefficient Experiments

Octanol-water partition coefficients were determined for SMX, ERY, and CIP as a function of pH (6, 7, or 8), calcium hardness (65 or 185 mg $CaCO_3$/L), and NOM (Table 3.3). ERY consistently exhibited the greatest logK_{ow} values. The logK_{ow} values for ERY increased with increasing pH but were unaffected by changes in hardness and NOM concentration. For SMX, logK_{ow} decreased with increasing pH. At pH 7 and 8, the SMX concentration in the octanol phase was below the analytical detection limit (0.25 mg/L). Thus, a value of (<-0.59) for these conditions is reported in Table 3.3. The logK_{ow} values for CIP were unaffected by the variation in pH, hardness, and NOM

Table 3.3. Effect of of pH, hardness, and NOM on the octanol-water partition coefficient for three selected antibiotics.

pH	hardness (mg $CaCO_3$/L)	NOM (mg C/L)	logK_{ow}		
			SMX	CIP	ERY
6	65	0.5	0.05	-0.43	0.44
	65	-	0.20	-0.43	0.38
	185	0.5	0.11	-0.44	0.41
	185	-	0.09	-0.45	0.39
7	65	0.5	<-0.59	-0.40	0.95
	65	-		-0.39	0.97
	185	0.5		-0.40	1.02
	185	-		-0.40	0.96
8	65	0.5	<-0.59	-0.41	1.66
	65	-		-0.40	1.59
	185	0.5		-0.42	1.66
	185	-		-0.43	1.64

3.4 Discussion

The sorption of antibiotics to biofilm was observed over a range of environmentally-relevant concentrations in this study. In general, the reproducibility in K_{oc} values for replicate runs was reasonable for a biological system. There were, however, considerable differences between K_{oc} values determined for ERY (2,000 and 8,000 L/kg) and SMX (0 and 4,000 L/kg) in replicate runs when fed in a mixture at high concentration (3.33 μg/L). This discrepancy might be due to variations in biofilm quantity, composition, or both between runs. The K_{oc} values were not affected by changes in antibiotic feed conditions (*i.e.*, fed alone or in a mixture at low and high concentrations), suggesting that inter- and intra-species competition for sorption sites was not a factor in these experiments. Thus, all of the values for a given antibiotic were used to compute a mean K_{oc} value.. The relative standard errors (*i.e.* standard error/mean) for the mean K_{oc} values (11% to 25%)

are comparable to those reported by Wicke *et al*. (2007) for compound sorption to biofilm.

The sorption rate constant (k) is an effective sorption rate that reflects the difference between sorption and desorption rates (Headley *et al*., 1998). There was no attempt to isolate the two rates in this investigation. Reported k values for the sorption of organic pesticides to biofilm (Headley *et al*., 1998) were of the same order of magnitude (0.05 to 3.6 hr^{-1}) and with similar variability between replicates runs as observed in this work. The k values ERY and SMX were similar but much lower for CIP. The significantly different K_{oc} and k values for CIP suggests that there may be a difference between the sorption mechanism for CIP and the other two antibiotics. More work is needed to investigate antibiotic sorption to biofilm to elucidate the mechanisms and sites involved.

In developing the mass balance model describing the fate of antibiotics in the CFRAB, it was assumed that biodegradation was negligible. The assumption was verified by the approach of the effluent antibiotic concentration to the feed concentration (*i.e*., C/C_{feed} = 1) in the sorption experiments (Figure 3.2). The lack of biodegradation in our system is not surprising as previous studies have shown that the selected antibiotics biodegrade slowly if at all and the duration of each sorption experiment in this work was only 48 hours. For example, ERY was not biodegraded at mg/L concentrations in wastewater treatment plant effluent with 40-day closed-bottle tests (Alexy *et al*., 2004), while Gavalchin and Katz (1994) reported slow ($t_{1/2}$ of 11.5 days) aerobic biodegradation in soil. Published results concerning SMX biodegradation are mixed. SMX was not

aerobically biodegraded in soil (Gavalchin and Katz, 1994), but sulfonamides, including SMX, were degraded by activated sludge ($t_{1/2}$ of 0.2 to 3 days) following 7-10 days of acclimation at 20°C under aerobic conditions (Ingerslev and Halling-Sorensen, 2000). Drillia *et al*. (2005a) also observed biodegradation of SMX by aerobic activated sludge biomass but only in the absence of another carbon source or nitrogen source, or both. Finally, a half-life of ~2-days was reported for CIP in activated sludge (Rabolle and Splid, 2000), but Al-Ahmad *et al*. (1999) reported that CIP was inhibitory to wastewater bacteria at concentrations as low as 80 μg/L.

The K_{oc} values determined in this study compare favorably with the K_{oc} values reported by others for antibiotic sorption to various sorbents (Table 3.4); mimicking the relative order of K_{oc} (CIP>ERY>SMX). Although our average K_{oc} value for ERY (6,000 ± 1,000 L/kg) is lower than reported K_{oc} values for sediment (30,600 and 50,550 L/kg), others studying the fate of ERY in activated sludge processes have reported no sorption of ERY to biomass (Li and Zhang, 2010). Our mean K_{oc} values for SMX (4,000 ± 1,000 L/kg) and CIP (92,000 ± 10,000 L/kg) are within the ranges of reported K_{oc} values for SMX (114 to 13,350 L/kg) and CIP (61,000 and 320,000 L/kg).

We are aware of only a few studies concerning the sorption of organic compounds to biofilm (Headley *et al.*, 1998; Wicke *et al*., 2007) and the compounds used in those studies (*i.e*. pesticides and polycyclic aromatic hydrocarbons) were considerably more hydrophobic than the antibiotics used in this research. For relatively hydrophobic

compounds ($\log K_{ow} > 1.7$), the biofilm K_{oc} values are in reasonable agreement with a correlation developed by Baker *et al*. (1997) for sorption of organic compounds to soil

Table 3.4. K_{oc} values for sorption of ERY, SMX, and CIP to organic matter of a biofilm compared to other organic sorbents (literature data).

Antibiotic	this study	other studies			
	K_{oc} (L/kg)	K_{oc} (L/kg)	sorbent	pH of water	Reference
ERY	6,000 ± 1,000	30,600[A]	Sediment	6.5-7.5	Kim and Carlson, 2007
		50,550[B]	Marine sediment	not specified	Xu *et al*., 2009
SMX	4,000 ± 1,000	530[C]	Soil	4.3	Drillia *et al*., 2005b
		114-2951	Digested sludge	5.5-6.6	Carballa *et al*., 2008
		674[D]	Activated sludge	7.0-7.5	Göbel *et al*., 2005
		13,350[C]	Marine sediment	not specified	Xu *et al*., 2009
CIP	92,000 ± 10,000	61,000[E]	Soil	5.0	Nowara *et al*., 1997
		320,000[F]	Sediment	7.5	Belden *et al*., 2007

[A] From mean of soil organic matter (0.69%) and Pseudo K_d (211 L/kg): mean of 10 sample events: ratio of measured concentration in sediment to water concentration in overlying river. K_{oc} determined by considering mean value for organic matter (method not provided)
[B] Reported value of K_{oc} in dynamic flume experiment
[C] Batch isotherms (0.01M $CaCl_2$). soil with 7.1% organic carbon (OC)
[D] From K_d, assuming 38% OC in TSS. K_d determined from ratio of the sorbed and dissolved fraction measured. b Calculated using concentrations from weekly sampling campaigns (dissolved fraction,n)3; sorbed fraction,n)1).
[E] Sandy soil with 0.7% OC. Reported pH not directly given, but assumed based on reported conditions for similar tests for enrofloxacin.
[F] From K_d, converted to K_{oc} using reported value of OC in sediment of 5.19 %

organic carbon (Figure 3.3), suggesting that: (1) the sorptive behavior of biofilm is similar to that of soil organic matter and (2) $\log K_{ow}$ is a useful predictor of the sorption of hydrophobic compounds to biofilm.

The hydrophilic antibiotics in this work ($\log K_{ow} < 1.7$), however, were not consistent with the Baker *et al*. (1997) correlation. Similarly, the sorption of antibiotics to manure (Loke *et al*., 2002) and digested sludge (Carballa *et al*., 2008) also did not correlate with $\log K_{ow}$. This result is not surprising as the hydrophilic organic compounds ($\log K_{ow}$ <

1.7) in the Baker *et al*. (1997) study were not consistent with the correlation. The reason for the different behavior of these low $\log K_{ow}$ compounds is that the sorption of

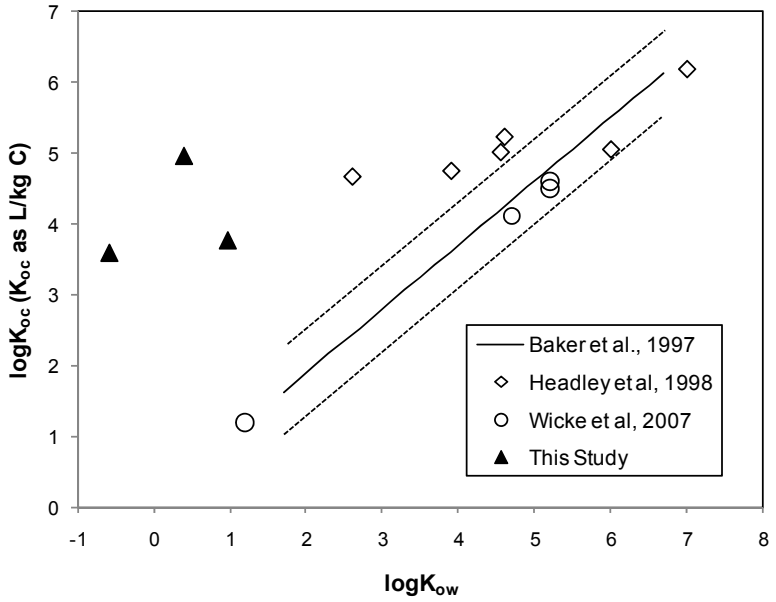

Figure 3.3. logK_{oc} versus logK_{ow} for organic compounds and biofilm and comparison with a correlation developed by Baker *et al*. for sorption of organic compounds to soil organic carbon. The solid line represents the correlation equation (logK_{oc} = 0.903logK_{ow}+0.094) and the dashed lines represent the 90% confidence intervals. For the antibiotics data from this study, the logK_{ow} values are at pH 7.

hydrophilic compounds is not controlled by hydrophobic interactions, predicted by partitioning into octanol, but other types of interactions (*e.g.*, ionic). Thus, in order to understand the partitioning behavior of antibiotics into biofilm, the potential for ionic interactions needs to be explored.

The physicochemical character of bacterial biofilm is controlled by the chemistry of the extracellular polymeric substances (EPS). EPS include proteins (measured from 10 to

82% by weight of total extracted EPS), humic substances (30-60%), carbohydrates (7-30%), uronic acids (3-22%), and DNA (2-15%) (Jahn and Nielsen 1995, Flemming *et al*., 1998). Heterotrophic EPS exhibits pK_A values associated with carboxylic (5.8 to 7.6) and phenolic or amino (8.4 to 9.5) functional groups, with an isoelectric point for EPS of 7.3 to 7.7 (Lee and Davis, 2001; Guibaud *et al*., 2003). Because EPS contains functional groups that are anionic (*e.g.*, $-COO^-$, $-SH^-$, $-SO_4^-$, $-HPO_4^-$) and cationic (*e.g.*, $-NH_3^+$), it has exchange potential for both cations and anions, and the apolar functional groups (*e.g.*, aromatic) of EPS proteins may sorb apolar organic compounds (Flemming *et al*., 1996). Uronic acids that comprise 20-50% of EPS polysaccharides provide high binding potential for cationic solutes (Kennedy and Sutherland, 1987). Although only a small fraction of the biofilm composition, bacterial cells also provide sorption sites. Cationic exchange occurs with anionic sites of the peptidoglycan and teichoic acid of gram positive bacteria, with anionic exchange from positively charged ammonium sites (Beveridge, 1984). Anionic sites of the peptidoglycan in the cell envelope of gram negative bacteria provide for cationic exchange (Beveridge and Koval, 1981). At pH > 7 EPS is generally negatively charged and hydrophilic (Bryers, 2000). Contact angles ranging from 15 to 37° have been reported for EPS from activated sludge with surface charges ranging from -0.41 to -0.21 meq/g VSS (Liao *et al*., 2001).

The functional groups of antibiotics greatly influence their activity in biological and chemical systems, with speciation dictated by compound pK_A values and solution pH (Figure 3.4). SMX exhibits pK_A values of 1.85 and 5.60 associated with the aromatic amine and the sulfonamide groups, respectively. SMX is anionic above pH 5.6. The pK_A

values of 3.0, 6.1, 8.7, and 10.6 for CIP are associated with the carboxylic acid group and the three nitrogen groups, respectively. Thus, between pH 6.1 and 8.7, CIP is predominantly cationic. ERY has a single pK_A of 8.9 associated with the dimethylamine and is predominantly cationic at pH 7. Consequently, the relative extent of sorption to biofilm (CIP>>ERY>SMX) is expected given the extent of species ionization for each

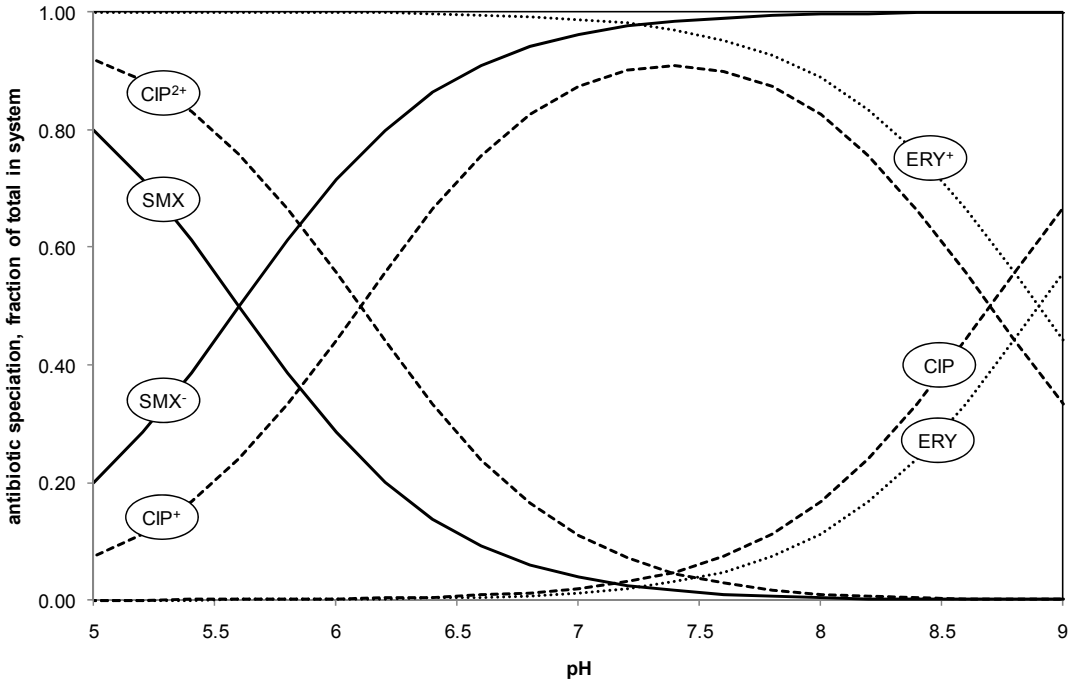

Figure 3.4. Antibiotic Speciation as a function of pH.

antibiotic. More sorption might be expected for ERY since it is predominantly protonated at circum-neutral pH. The reason for the greater extent of sorption for CIP as compared to ERY, which both exhibit comparable protonation at circumneutral pH, is unclear. The logK_{ow} values at pH 7 for ERY and CIP were 0.98 and -0.4, respectively which suggests that ERY is more hydrophobic and might sorb via both ionic and hydrophobic interactions.

One possible explanation is the decreased rate of mass transfer of ERY to and through the biofilm, as the molecular weight of ERY is more than twice that for CIP. Because of the effects of pH on antibiotic and EPS speciation, it is expected that fluctuations in system pH would affect the retention (or desorption) of antibiotics sorbed to biofilm. More research is needed to investigate the mechanism or mechanisms of antibiotic sorption to biofilm including the relevant sorption sites as well as the potential for antibiotic desorption from biofilms.

3.5 Conclusions

In this study, the biofilm organic carbon sorption coefficients for three selected antibiotics (ERY, SMX, and CIP) were determined using a CFRAB reactor. The main conclusions of this work are as follows:

- The rate and extent of antibiotic sorption to biofilm is dependent on antibiotic structure. CIP exhibited a greater extent (K_{oc}) and lower rate (k) of sorption compared to ERY or SMX.
- the K_{oc} values describing antibiotic partitioning to biofilm did not correlate with the antibiotic K_{ow} values suggesting that hydrophobic interactions are not important for sorption of these relatively hydrophilic compounds to biofilm
- antibiotic speciation and molecular size are important for explaining the interaction between antibiotics and biofilm.

The results of this work may be useful for:

- modeling the fate of antibiotics in biologically-active filtration systems used for drinking water production, including slow sand filtration, bank filtration, and rapid filtration;
- predicting the fate of antibiotics in other systems where biofilms are present including: drinking water distribution networks, aquifer recharge systems, soil-aquifer treatment installations, wastewater fixed-film bioreactors, and septic system leach fields; and
- selecting or developing antibiotics for inactivation of bacterial biofilms.

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3.6 References

Adams, C., Wang, Y., Loftin, K., and Meyer, M. (2002) Removal of antibiotics from surface and distilled water in conventional water treatment processes, Journal of Environmental Engineering 128(3), 253-260.

Al-Ahmad, A., Daschner, F.D., Kummerer, K. (1999) Biodegradability of Cefotiam, Ciprofloxacin, Meropenem, Penicillin G, and Sulfamethoxazole and inhibition of waste water bacteria. Archive of Environmental Contamination and Toxicology 37(2), 158-163.

Alexy, R., Kümpel, T., Kümmerer, K. (2004) Assessment of degradation of 18 antibiotics in the Closed Bottle Test.Chemosphere 57(6), 505-512.

APHA. (1995) *Standard Methods for the Examination of Water and Wastewater*, 19th Ed. American Public Health Association, Washington, D.C.

Ash, R.J., Mauck, B., Morgan, M. (2002) Antibiotic resistance of gram negative bacteria in rivers, United States. Emerging Infectious Diseases 8(7), 713-716.

Baker, J.R., Mihelcic, J.R., Luehrs, D.C., Hickey, J.P. (1997) Evaluation of estimation methods for organic carbon normalized sorption coefficients. Water Environment Research 69(2), 136-145.

Belden, J.B., Maul, J.D., Lydy, M.J. (2007) Partitioning and photodegradation of ciprofloxacin in aqueous systems in the presence of organic matter Chemosphere 66, 1390–1395.

Benotti, M.J., Trenholm, R.A., Vanderford, B.J., Holady, J.C., Stafford, B.D., Snyder, S.A. (2009) Pharmaceuticals and endocrine disrupting compounds in US drinking water. Environmental Science &Technology 43(9), 597-603.

Beveridge, T.J., Koval, S.F. (1981) Binding of metals to cell envelopes of Escherichia coli K-12. Applied Environmental Microbiology 42(2), 325-335.

Beveridge, T.J. (1984) Bioconversion of inorganic materials: mechanisms of the binding of metallic ions to bacterial walls and the possible impact on microbial ecology. In: Klug, M.J.; Reddy, C.A. (eds) Current perspectives in Microbial Ecology. American Society of Microbial Ecology, Washington, D.C. pp 601-607.

Bouwer, E.J.,Crowe, P.B. (1988) Biological processes in drinking water treatment. Journal AWWA 80(9), 82-93.

Bryers, J. (Ed.). (2000) *Biofilms II-Process Analysis and Applications*. Wiley, New York, NY.

Carballa, M., Fink, G., Omil, F., Lema, J.M., Ternes, T. (2008) Determination of the solid-water distribution coefficient (K_d) for pharmaceuticals, estrogens, and musk fragrances in digested sludge. Water Research 42(1-2), 287-295.

Chamberlain, E.,Adams, C. (2006) Oxidation of sulfonamides, macrolides, and carbadox with free chlorine and monochloramine. Water Research 40(13), 2517-2526.

Collins, M.R., Eighmy, T.T., Fenstermacher, J.M., Spanos, S.K (1989) Modifications to the Slow Sand Filtration Process For Improved Removals of Trihalomethane Precursors. AWWARF, Denver, CO.

Congliang, Z., Yan, W., Fuan, W. (2007) Determination and Temperature Dependence of *n*-Octanol/Water Partition Coefficients for Seven Sulfonamides from (298.15 to 333.15) K. Bulletin of the Korean Chemical Society 28(7), 1183-1186.

Dodd, M.C., Huang, C-H. (2004) Transformation of the antibacterial agent sulfamethoxazole in reactions with chlorine: kinetics, mechanisms, and pathways. Environmental Science & Technology 38(21), 5607-5615.

Drakapoulos, A.I., Ioannou, P.C. (1997) Spectrofluorimetric study of the acid-base equilibria and complexation behavior of the fluoroquinolone antibiotics ofloxacin, norfloxacin, ciprofloxacin and pefloxacin in aqueous solution. Analytica Chemistry Acta 354(1), 197-204.

Drillia, P., Dokianakis S.N., Fountoulakis M.S., Kornros M., Stamatelatou K., Lyberatos G. (2005a) On the occasional biodegradation of pharmaceuticals in the activated sludge process: The example of the antibiotic sulfamethoxazole. Journal of Hazardous Materials 122(3), 259- 265

Drillia, P., Stamatelatou, K., Lyberatos, G. (2005b) Fate and mobility of pharmaceuticals in solid matrices. Chemosphere 60, 1034–1044.

Eighmy, T.T., Collins, M.R., Malley, J.P., Royce, J., Morgan, D. Biologically Enhanced (1993) Slow Sand Filtration for Removal of Natural Organic Matter. AWWARF, Denver, CO.

Flaherty, C.M., Dodson, S.I. (2005) Effects of pharmaceuticals on *Daphnia* survival, growth, and reproduction. Chemosphere 61(2), 200-207.

Flemming, H.-C., Schmitt, J., Marshall, K. C. (1996) In *Sorption properties of biofilms*. *Sediments Toxicology Substances*; Calmano, W., Foerstner, U., Eds.; Springer: Berlin, Germany, pp 115-157.

Gagnon, G.A., Huck, P.M.. 2001. Removal of easily biodegradable organic compounds by drinking water biofilms: analysis of kinetics and mass transfer. Water Research 35(10):2554-2564.

Gavalchin, J., Katz, S.E. (1994) The persistence of fecal-borne antibiotics in soil. Journal of the Association of Official Analytical Communities International 77(2), 481-485.

Ghosh, G.C., Okuda, T., Yamashita, N. (2009) Occurrence and elimination of antibiotics at four sewage treatment plants in Japan and their effects on bacterial ammonia oxidation. Water Science and Technology 59(4), 779-786.

Gjaltema, A., Arts, P.A.M., van Loosdrecht, M.C.M., Kuenen, J.G., Heijnen, J.J. (1994) Heterogeneity of biofilms in rotating annular reactors: occurrence, structure, and consequences. Biotechnology and Bioengineering 44(2), 194-204.

Göbel, A., McArdell, C.S., Suter, M.J-F., Giger, W. (2004) Trace determination of macrolide and sulfonamide antimicrobials, a human sulfonamide metabolite, and trimethoprim in wastewater using liquid chromatography coupled to electrospray tandem mass spectrometry. Analytical Chemistry 76(16), 4756.

Göbel, A., Thomsen, A., McArdell, C.S., Joss, A., Giger, W. (2005) Occurrence and sorption behaviour of sulfnonamides, macrolides and trimethoprim in activated sludge treatment. Environmental Science & Technology 39 (11), 3981–3989.

Guibaud, G., Comte, S., Bordas, F., Dupuy, S., Baudu, M. (2005) Comparison of the complexation potential of extracellular polymeric substances (EPS), extracted from activated sludges and produced by pure bacteria strains, for cadmium, lead and nickel. Chemosphere 59(5), 629-638.

Hansch, C., Leo, A., Hoekman, D. (1995) *Exploring QSAR: Hydrophobic, Electronic, and Steric Constants.* Vol. 2. American Chemical Society, Washington, D.C.
Halling-Sorensen, B. (2000) Algal toxicity of antimicrobial agents used intensive farming. Chemosphere 40(7), 731-739.

Headley, J.V., Gandrass, J., Kuballa, J., Peru, K.M., Gong, Y. (1998) Rates of Sorption and Partitioning of Contaminants in River Biofilm. Environmental Science & Technology 32(24), 3968-3973.

Hiscock, K.M., Grischek, T. (2002) Attenuation of groundwater pollution by bank filtration. Journal of Hydrology 266(3-4), 139-144.

Huber, M. M., Korhonen, S., Ternes, T. A., von Gunten, U. (2005) Oxidation of pharmaceuticals during water treatment with chlorine dioxide. Water Research 39(15), 3607-3617.

Ingerslev, F., Halling-Sørensen, B. (2000) Biodegradability properties of sulfonamides in activated sludge. Environmental Toxicology and Chemistry 19(10), 2467-2473.

Kennedy A.F., Sutherland I.W. (1987) Analysis of bacterial exopolysaccharides. Biotechnology and Applied Biochemistry 9(1), 12–19.

Kim, S-.C., Carlson, K. (2007) Temporal and spatial trends in the occurrence of human and veterinary antibiotics in aqueous and river sediment matrices. Environmental Science & Technology 41(1), 50-57.

Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., Buxton, H.T. (2002) Pharmaceuticals, hormones, and other organic wastewater constituents in US streams, 1999-2000: a national reconnaissance. Environmental Science & Technology 36(6), 1202-1211

Lee, S.M., Davis, A.P. (2001) Removal of Cu (II) and Cd (II) from aqueous solution by seafood processing waste. Water Research 35(2), 534-540.

Li, B., Zhang, T. (2010) Biodegradation and Adsorption of Antibiotics in the Activated Sludge Process Environmental Science & Technology *article ASAP*: DOI: 10.1021/es903490h

Liao, B.Q., Allen, D.G., Droppo, I.G., Leppard, G.G., Liss, S.N. (2001) Surface Properties of sludge and their role in bioflocculation and settleability. Water Research 35(2), 339-350.

Lindberg, R., Jarnheimer, P-A., Olsen, B., Johansson, M.I., Tysklind, M. (2004) Determination of antibiotic substances in hospital sewage water using solid phase extraction and liquid chromatography/mass spectrometry and group analogue internal standards. Chemosphere 57(10), 1479-1488.

Loke, M.L., Tjornelund, J., Halling-Sorensen, B. (2002) Determination of the distribution coefficient (log K_d) of oxytetracycline, tylosin A, olaquindox and metronidazole in manure. Chemosphere 48(3), 351-361.

McArdell, C.S., Molnar, E., Suter, M.J., Giger, W. (2003) Occurrence and fate of macrolide antibiotics in wastewater treatment plants and in the Glatt Valley watershed, Switzerland. Environmental Science and Technology 37(24): 5479.

McFarland, J.W., Berger, C.M., Froshauer, S.A., Hayashi, S.F., Hecker, S.J., Jaynes, B.H., Jefson, M.R., Kamicker, B.J., Lipinski, C.A., Lundy, K.M., Reese, C.P., Vu, C.P. (1997) Quantitative Structure−Activity Relationships among Macrolide Antibacterial Agents: In Vitro and in Vivo Potency against *Pasteurella multocida.* Journal of Medicinal Chemistry 40(9), 1340-1346.

Moore, D.E., Zhou, W. (1994) Photodegradation of sulfamethoxazole: A chemical system capable of monitoring seasonal changes in UVB intensity. Photochemistry and Photobiology 59(5), 497-502.

Ngheim, L.D., Schäfer, A.I., Elimelech, M. (2005) Pharmaceutical retention mechanisms by nanofiltration membranes. Environmental Science & Technology 39(19), 7698-7705.

Nowara, A., Burhenne, J., Spiteller, M. (1997) Binding of fluoroquinolone carboxylic acid derivatives to clay minerals. Journal of Agricultural and Food Chemistry 45(4), 1459–1463.

Pena, A., Chmielova, D., Lino, C.M., Solich, P. (2007) Determination of fluorquinolone antibiotics in surface waters from Mondego River by high performance liquid chromatography using amonolithic column. Journal of Separation Science 30(17), 2924-2928.

Qiang, Z., Adams, C. (2004) Potentiometric determination of acid dissociation constants (pK_a) for human and veterinary antibiotics. Water Research 38(12), 2874-2890.

Rabolle, M., Spliid, N.H. (2000) Sorption and mobility of metronidazole, olaquindox, oxytetracycline and tylosin in soil. Chemosphere 40(7), 715-722.

Radjenovića, J., Petrović, M., Barcelóa, D., Petrović, M. (2007) Advanced mass spectrometric methods applied to the study of fate and removal of pharmaceuticals in wastewater treatment. Trends in Analytical Chemistry 26(11), 1132-1144.

Renew, J, Huang, C.H. Simultaneous determination of fluoroquinolone, sulfonamide, and trimethoprim antibiotics in wastewater using tandem solid phase extraction and liquid chromatography-electrospray mass spectrometry." Journal of Chromatography A, 2004, 1042: 13.

Scribner, E.A.; Battaglin, W.A.; Dietze, J.E.; Thurman, E.M.. Reconnaissance data for glyphosphate, other selected herbicides, their degradation products, and antibiotics in 51 streams in nine midwestern states, 2002. U.S. Geological Survey: Lawrence, Kansas

Snyder, S.A., Adham, S., Redding, A.M., Cannon, F.S., DeCarolis, J., Oppenheimer, J., Yoon, Y. (2007) Role of membranes and activated carbon in the removal of endocrine disruptors and pharmaceuticals. Desalination 202(1-3), 156-181.

Thiele-Bruhn, S. (2003) Pharmaceutical antibiotic compounds in soil – a review. Journal of Plant Nutrition and Soil Science 166(2), 145-167.

Torniainen, K., Mattinen, J., Askolin, C.P., Tammilehto, S. (1997) Structure elucidation of a photodegradation product of ciprofloxacin. Journal of Pharmaceutical and Biomedical Analysis 15(7), 887-894.

USEPA. (1996) OPPTS 830.7550 Partition Coefficient (n-octanol/water), shake flask method. EPA 712-C-96-038, Washington, D.C.

Weiss, W.J., Bouwer, E.J., Ball, W.P., O'Melia, C.R. Arora, H., Speth, T.F. (2003) Comparing RBF with bench-scale conventional treatment for precursor reduction. Journal of the American Waterworks Association 95(12), 67-80.

Westerhoff, P., Yoon, Y., Snyder, S., Wert, E. (2005) Fate of endocrine-disruptor, pharmaceutical, and personal care product chemicals during simulated drinking water treatment processes. Environmental Science & Technology, 39(17), 6649-6663.

Wicke, D., Böckelmann, U., Reemtsma, T. (2007) Experimental and modeling approach to study sorption of dissolved hydrophobic organic contaminants to microbial biofilms. Water Research 41(10), 2201-2210.

Xu, W.H., Zhang, G., Wai, O.W.H., Zou, S.C., Li, X.D. (2009) Transport and adsorption of antibiotics by marine sediments in a dynamic environment. Journal of Soils and Sediments 9, 364–373.

Yang, Li-.H., Ying, G-.G., Su, H-,C., Stauber, J.L., Adams, M.S., Binet, M.T. (2008) Growth-inhibiting effects of 12 antibacterial agents and their mixtures on the freshwater microalga *Pseudokirchneriella subcapitata*. Environmental Toxicology and Chemistry 27(5), 1201–1208.

Zabinski, R.A.; Walker, K.J.; Larsson, A.J.; Moody, J.A.; Kaatz, G.W.; Rotshafer, J.C. Effect of aerobic and anaerobic environments on antistaphylococcal activities of five fluoroquinolones. Antimicrobial Agents and Chemotherapy, 1995, 39:2:507-512.

Chapter 4. Bioattentuation and Effect of Antibiotics in Slow-Rate Biofiltration Processes

Antibiotics are detected in surface waters worldwide, but little is known about their fate in slow-rate biofiltration process (SRBF) such as slow sand filtration (SSF) and riverbank filtration (RBF), or their potential impacts on system bacteria. In this research, the attenuation and impact of antibiotics in slow-rate biofiltra tion systems was investigated. Bench-scale filter column experiments and batch sorption studies were conducted with a mixture of sulfamethoxazole (SMX), erythromycin (ERY), and ciprofloxacin (CIP) at high (3.33 μg/L, each) and low (0.33 μg/L, each) antibiotic concentrations. Antibiotic breakthrough times were greatest for CIP, with both SMX and ERY exhibiting very little retention in the bench-scale SRBF system. The experimental results compared well with results for modeled breakthrough derived from the one-dimension advection-dispersion equation (ADE) that accounts for linear sorption and ideal plug-flow behavior (*i.e.*, no dispersion). Based on ADE model results for typical SSF, RBF, and rapid gravity (bio)filtration systems (RGBF), there was no effective removal of antibiotics in RGBF, little removal expected for SSF, and some removal of antibiotics expected for RBF where retardation is greatest among the three systems. While non-metric multidimensional scaling of automated ribosomal intergenic spacer analysis data indicate that the biofilm community structure was not affected by antibiotics at either concentrations, minor impacts on individual species were observed for the high concentration antibiotic feed condition. The results of this research suggest that little removal of most antibiotics can be expected in SSF, and that mixtures of antibiotics at the sub-μg/L concentrations

typically observed in surface waters are unlikely to affect the community structure of biofilm bacteria critical to process performance.

4.1 Introduction

Antibiotics have been detected in surface waters worldwide, including North America, Europe, and Asia; with reported concentrations typically in the low ng/L range, but as high as 1.9 μg/L (Kolpin *et al*., 2002; Giger *et al*, 2003; Wiegel *et al*., 2004; Kim *et al*., 2007). Their environmental persistence is partially due to the very properties that give them value in medicine. Designed for mobility in animal body tissue, antibiotics are usually hydrophilic (*i.e*., low logK_{ow}) and charged at circumneutral pH. Low levels of antibiotics could promote the development of antibiotic resistant bacteria Also, mixtures of antibiotics at low concentrations (*i.e*., 0.1 to 50 μg/L) can adversely affect algae, nitrifying bacteria, and zooplankton (Flaherty and Dodson, 2005; Yang *et al*., 2008; Ghosh *et al*., 2009). Though human health effects of sustained antibiotic exposure (at sub-therapeutic doses) are unknown, public awareness over the presence of antibiotics and other pharmaceutical compounds in drinking water supplies is heightened (Benotti *et al*., 2009). Thus, there is significant interest in approaches to remove antibiotics and other pharmaceutical compounds from water supplies.

Conventional water treatment (*i.e*., coagulation/flocculation/sedimentation/filtration) andr lime softening do not effectively remove antibiotics (≤33%, Adams *et al*., 2002; Westerhoff *et al*., 2005). Numerous antibiotics, however, are transformed by various disinfection/oxidation processes. For example, removals of sulfonamides, carbodox, and

trimethoprim in surface water exposed to free chlorine (1 mg/L for 40 minutes) exceeded 90%, although sulfamethoxazole (SMX) may reform during dechlorination (Adams *et al*., 2002; Dodd and Huang, 2004). Chlorine dioxide and ozone also effectively remove antibiotics (*e.g.*, sulfonamides and macrolides) at feasible doses and contact times (Huber *et al*., 2005; Westerhoff *et al*., 2005), but this is not the case for chloramines (Chamberlain and Adams, 2006). Fresh granular activated carbon (GAC) effectively removes SMX and erythromycin (ERY) at reasonable empty-bed contact times, with spent GAC still exhibiting some removal (<40% for SMX and <55% for ERY) (Westerhoff *et al*., 2005; Snyder *et al*., 2007). Among membrane filtration alternatives only reverse osmosis and nanofiltration effectively reject antibiotics (Ngheim *et al*., 2005; Snyder *et al*., 2007).

Slow-rate biofiltration processes (SRBF), such as slow sand filtration (SSF) and riverbank filtration (RBF), have been used in Europe for more than 100 years, and there is increasing interest in these systems in the US because of deteriorating surface water quality and stricter drinking water regulations. In SRBF systems, water flows through an engineered or natural support media (*e.g.*, sand, engineered media, or aquifer material) colonized by bacteria residing in biofilms, with typical filtration rates (and residence times) of 0.1 to 0.2 m/hr (2 to 4 hours) for SSF and <0.1m/hr (5 to 100 days) for RBF (Kawamura, 1991; Kuehn and Mueller, 2000).

SRBF systems remove pathogens and organic compounds such as disinfection byproduct precursors, pesticides, and pharmaceutical compounds (Weber-Shirk and Dick, 1997;

Collins and Eighmy, 1989; and Hiscock and Grischek, 2002). Recent work has focused antibiotic removal in SSF and RBF. The modeling results of Rooklidge *et al.* (2005) that predicted 3-log removal of macrolide and quinolone antibiotics in SSF are contrasted by the results (26% removal of SMX and no removal of ERY) from a study of full-scale SSF studies used for tertiary wastewater (Nakada *et al.*, 2007). Heberer *et al.* (2008) reported removals of ERY (90%) and SMX (52 and 99% under oxic and anoxic conditions, respectively) in full-scale RBF, and indicated that these antibiotics were the most persistent among the antibiotics studied. Similar SMX removals in full-scale RBF were reported by Grünheid *et al*. (2005). Unfortunately, these researchers did not attempt to elucidate the dominant mechanisms of antibiotic removal (*i.e*. sorption versus degradation) or the impact of the antibiotics on the biofilm bacteria.

Although removal of antibiotics from the water supply may be the desired outcome, there is some concern that the antibiotics may be inhibitory to the biofilm bacteria that are critical to SRBF system performance. While there are numerous reports of bacterial inhibition at antibiotic concentrations ≥ 20 μg/L (Muñoz-Aguayo *et al*.,2007; Knapp *et al*., 2008; Ghosh *et al*., 2009), few studies have been performed to investigate the effects of antibiotics at environmentally-relevant concentrations on mixed-species bacterial biofilms. Shifts in community structure of biofilm bacteria when exposed to ciprofloxacin (CIP) at a concentration of 3.33 μg/L are reported in Chapter 2.

In this study, we describe the results of bench-scale filter column experiments designed to investigate antibiotic attenuation in SRBF processes during the start-up and maturation

phases. Furthermore, we also investigated the effect of antibiotic mixtures on biofilm development and bacterial community structure. Also, batch experiments were performed using schutzdecke material from the bench-scale filter columns and from a full-scale SSF to evaluate the importance of sorption and biodegradation to antibiotic attenuation in SSFs.

4.2 Materials and Methods

4.2.1 Experimental Design

The fate and impact of three antibiotics in slow-rate biofiltration systems was investigated using packed-bed columns and batch reactors. The aim of this research was to better understand the fate of antibiotics in SRBF systems and to identify the potential impacts on bacteria in biofiltration systems exposed to mixtures of antibiotics at low concentrations.

Three antibiotics, SMX, ERY, and CIP, were selected for this investigation for the following reasons, they: (1) represent three prominent classes of antibiotics; (2) have been detected in surface water; (3) exhibit different chemical characteristics (*e.g.*, K_{ow}); and (4) have differing levels of biodegradability. More information on the selected antibiotics is provided in Chapters 2 and 3.

Multiple filter column experiments were run as follows: (1) no antibiotics (control); (2) the three antibiotics each at 3.33 μg/L; (3) the three antibiotics each at 0.33 μg/L; All filter column experiments were performed in duplicate. In addition, batch experiments

were conducted using schmutzdecke material harvested from the filter columns and from a full-scale SSF.

4.2.2 Chemicals and Reagents

SMX (≥98% purity), ERY (≥95% purity), CIP (≥98% purity), were purchased from Sigma-Aldrich (St. Louis, MO, USA). All antibiotic compounds were used as received.

Fresh mineral medium (pH 7.25 ± 0.3) was prepared (using distilled water) every four to five days and contained (per liter of reactor water): 43.8 mg/L K_2HPO_4, 17 mg/L KH_2PO_4, 62.4 mg/L Na_2HPO_4, 45.0 mg/L $MgSO_4$, 0.5 mg/L $FeCl_3$-$6H_2O$, 5.4 mg/L NH_4Cl, and 55 mg/L $CaCl_2$. The acetate feed solution was prepared daily by dissolving sodium acetate in distilled water. All salts used to prepare the mineral medium were reagent grade (Sigma Aldrich, St. Louis, MO).

4.2.3 Column Experiments

The column experiments were conducted to evaluate antibiotic removals and to investigate the temporal and spatial (*i.e.*, depth) variation of biofilm accumulation and community composition. A schematic diagram of the filter column apparatus is provided in Figure 4.1. The polycarbonate filter columns had inside and outside diameters of 7.0 and 7.5 cm, respectively, and included five flanged sections (four of which were filled with ~300 g of dry sand), each 5 cm high, with top and bottom flanges. Prior to installation in the columns, the filter sand (Fairmount Minerals, Chardon, OH), with an effective size of 0.31 mm and a uniformity coefficient of 1.35, was placed in in a muffle

furnace for two hours at 550°C to eliminate any organic material. Each sand-filled section contained a stainless steel mesh (0.25 mm openings) on the bottom to facilitate removal for sand sampling at various depths to quantify biomass levels over time. Prior to each

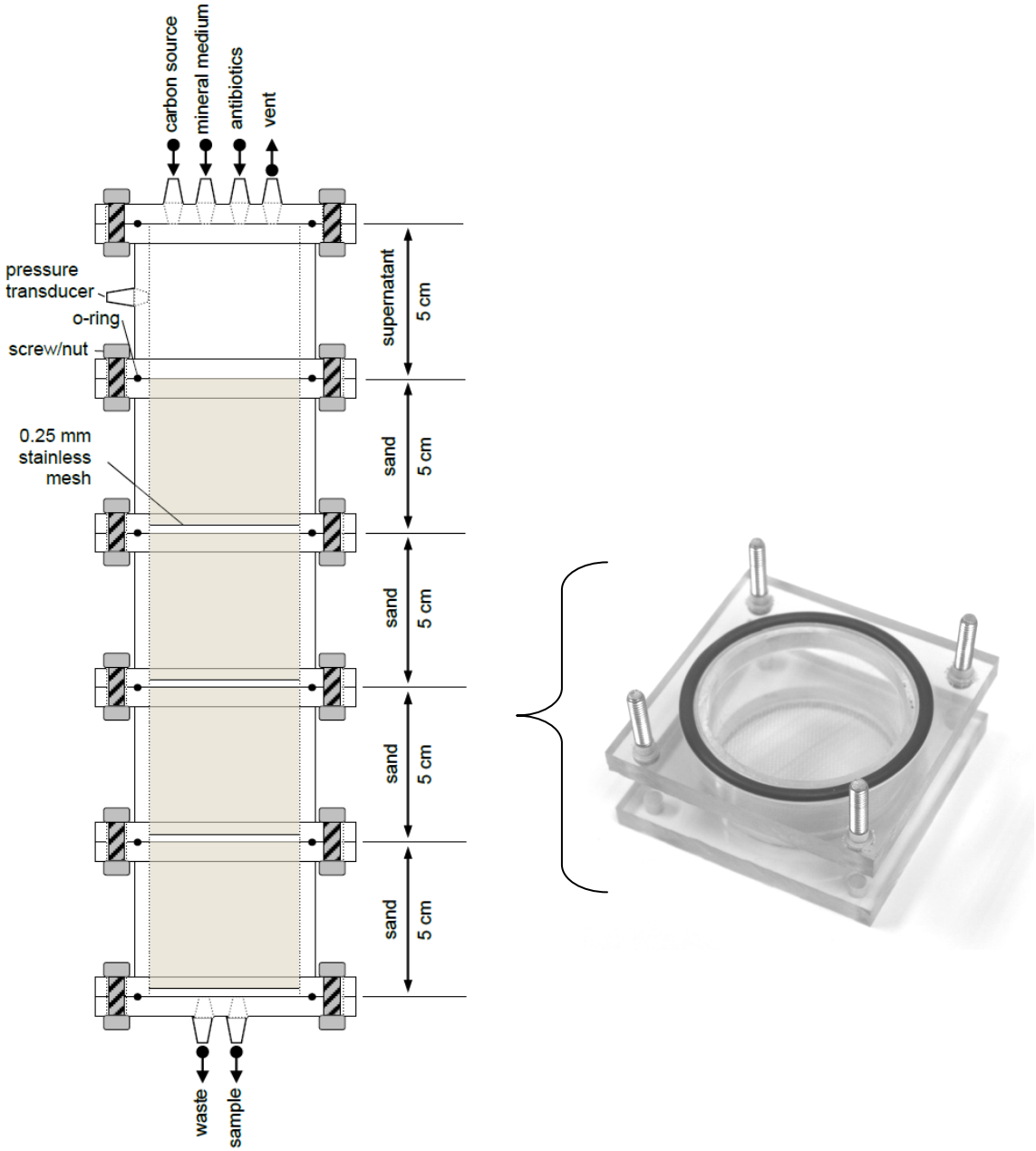

Figure 4.1 Schematic diagram of the filter column. Photo of single segment shown.

experiment the filter columns were disinfected by soaking for 24-hours with a 10% solution of NaOCl, then rinsed three times with deionized water, loaded with sand media and assembled, followed by a 24-hour rinse with deionized water at experimental loading rates. The filter columns were fed at a loading rate of 0.095 m/hr, including mineral medium (composition described earlier) and a combined feed of acetate (2 mg C/L) and antibiotics at either 0 mg/L (control), 0.33 μg/L, or 3.33 μg/L. The mineral medium and acetate/antibiotic feed were delivered to the column through peroxide-cured silicone tubing (Cole-Parmer) using peristaltic pumps. Fresh antibiotic/acetate feed solutions were made daily, while the inorganic minimal medium solution was made as needed (approximately once per week). Antibiotic/acetate feed solutions were used within 48 hours of preparation and stored at 4˚C prior to use. Pressure at the filter column inlet (*i.e.*, in the top column segment) was measured every hour with a transducer (Cole-Parmer EW-68075-40).

The filters were inoculated by injecting 10 mL of thawed cryopreserved biomass (revived by incubating with acetate and in mineral medium) into the top of the first filter column section using a sterile syringe. The inoculum was prepared from schmutzdecke of full-scale slow sand filters at the Metropolitan District in Hartford, CT (MDC). Schmutzdecke collection, crypreservation and inoculum methods are provided in Chpater 2. The feeding of mineral medium, acetate, and antibiotics (except for the control) commenced immediately after filter innoculation. The filter columns were operated at room temperature (23.7 ± 1.7 °C) for six weeks, with filtrate collected approximately three times per week (in 1 liter amber bottles) for analysis of antibiotics. Approximately 3

grams of filter sand (dry weight) were removed from each column section after two-, four-, and six weeks of operation for analysis of volatile solids (VS) and microbial community structure (via ARISA).

4.2.4 Batch Experiments

Batch experiments were conducted to investigate the sorption of antibiotics to schmutzdecke material, and to determine if antibiotics can be biodegraded by schmutzdecke bacteria. For these experiments, approximately 1 L of schmutzdecke from an MDC slow sand filter was shipped to Calvin College (via overnight on-ice) within 24-hours of collection. Upon receipt, half of the schmutzdecke material was shipped overnight (on ice) to an irradiation facility (Sterigenics, Gurnee, IL, USA) where it was sterilized via gamma irradiation (dose of 50 kGy). Batch experiments commenced upon receipt of non-sterilized and sterilized schmutzdecke material.

Each batch reactor consisted of a 1 liter amber bottle into which was added 900 mL of filter-sterilized (5.0 μm and 0.22 μm polypropylene Calyx Capsules in series, GE Water and Process Technologies, Westborough, MA, USA) water from Lake Michigan spiked with 100 μg/L each of SMX, CIP, and ERY, along with varied masses (0.5, 1.0, 2.0, 4.0, 8.0, and 16 g) of wet schmutzdecke material (to develop sorption isotherms). Duplicate batch reactors were placed on an orbital shaker table (120 rpm) for a 48-hour equilibration period. After incubation, the 900 mL aqueous samples were filtered (0.45 μm glass fiber filter, Millipore, Billerica, MA, USA) to remove schmutzdecke material and then stored for up to 2 days at ~4°C prior to analysis for antibiotics. The filtered

schmutzdecke material was retained for subsequent analysis of biomass as VS. All batch experiments were conducted in the dark at room temperature (23.7 ± 1.7°C).

4.2.5 Analytical Methods

4.2.5.1 Antibiotics

Antibiotics in aqueous solution were analyzed using a solid-phase extraction liquid chromatography-mass spectrometry (LC-MS) method described in Chapter 3.

4.2.5.2 Biomass as Volatile Solids

The amount of biomass in filter media and schmutzdecke was determined as volatile solids. Samples (~ 3 g wet weight) were placed in aluminum weighing trays. The samples were dried to a constant weight at 105 °C and then combusted at 550 °C for 2 hours. Volatile solids (VS) was computed as the difference between dry weight and post-combustion weight and then normalized to the mass of inert material (*i.e.*, post-combustion weight of the sample).

4.2.5.3 Community Structure

ARISA was used to assess the effects of antibiotics on community structure. Details on the ARISA procedure are provided in Chapter 2.

4.2.6 Data Analysis

4.2.6.1 Isotherms

The mass of antibiotic sorbed to schmutzdecke material (q) was determined by computing the difference between the total mass of antibiotic in the control (no schmutzdecke material) and the total mass at equilibrium in solution and then dividing by the mass of schmutzdecke material (as VS). The sorption data were fit to a linearized version of the Freundlich isotherm ($q = KC$) where K is the antibiotic sorption coefficient and C is the concentration of antibiotic in solution at equilibrium with the schmutzdecke material. The organic carbon sorption coefficient (K_{oc}) was calculated by dividing K by fraction of organic carbon (f_{oc}). A f_{oc} of 0.49 was estimated for schmutzdecke by assuming that VS was comprised of bacterial biomass with a chemical formula of $C_5H_7O_2N$.

4.2.6.2 Community structure

The peak area of each fragment was normalized by the total area of all fragments and expressed as a percentage. Fragments comprising less than 1% of the total area were excluded from the analysis. Non-metric multidimensional scaling (nMDS) was employed to evaluate the effect of antibiotic exposure on community structure using the R software program (R Development Core Team, 2009).

4.2.6.3 Modeling antibiotic breakthrough

A one-dimension advection-dispersion equation (ADE) that accounts for linear sorption was used to model antibiotics attenuation in the bench-scale SSF columns:

$$v_x \frac{dC}{dx} = \frac{dC}{dt} R_f \quad (1)$$

where: v_x is the water velocity in the filter, C is the antibiotic concentration at some time (t) and position (x), and R_f is the retardation factor, which represents the ratio of average water velocity to the average velocity of antibiotic attenuated by sorption to system media, given by:

$$R_f = 1 + \frac{\rho_b K}{\varepsilon} \quad (2)$$

where: ε is the system porosity, and ρ_b is the bulk density of system media. Assuming plug-flow conditions with no dispersion, the integrated form of equation 1 is used to determine breakthrough time, t_b, for each antibiotic;

$$t_b = \frac{x}{v_x} R_f \quad (3)$$

where: x is the travel distance.

The bench-scale SSF system was modeled as schmutzdecke (2.5 cm deep) in series with an abiotic filtration zone (17.5 cm deep). Model parameters were as follows: loading rate of 0.095 m/hr, porosity of 0.4, and a media bulk density (ρ_b) of 2.6 kg/L. The f_{oc} values for the schmutzdecke and filtration zone were estimated from measured VS values, assuming that carbon is 49% of VS by mass (*i.e.*, bacterial biomass with a chemical formula of $C_5H_7O_2N$). The values for K were obtained by multiplying the K_{oc} values for sorption of antibiotic to biofilm (as a surrogate for schmutzdecke) by the f_{oc} of the

schmutzdecke or abiotic filtration zone.Antibiotic-biofilm K_{oc} values reported in Chapter 3 were as follows: 92,000, 6,000, and 4,000 L/kg for CIP, ERY, and SMX, respectively.

4.3 Results

4.3.1 Effect of antibiotics on biofilm accumulation

Filter column biomass levels over time were determined indirectly via headloss (Figure 4.2) and directly as VS (Figure 4.3). There was considerably more headloss development for low antibiotic feed concentration run than that for high concentration feed conditions. While headloss increased (even after biomass sampling events at 14 and 28 days) through the duration of testing for the low feed condition, headloss development during the high feed concentration did not increase to previous levels after the 28-day sample event. For both experiments, headloss development was greatest for one of the antibiotic-fed columns, but similar trends were exhibited by the second antibiotic-fed column and both control columns. At zero depth (the water-media interface) and two weeks, the amount of biofilm in the low antibiotic feed concentration (0.33 μg/L each) columns (2.69 and 2.84 mg VS/g media) was similar to that in the control columns (2.42 and 2.57 mg VS/g), while at four weeks more biofilm VS was present in antibiotic-fed columns (4.71 and 5.31 mg VS/g media) than in the control columns (3.39 and 3.41 mg VS/g media). For columns receiving high concentrations of antibiotics (3.33 μg/L each), at two weeks there was similar biofilm VS quantities present for the two control columns (1.47 and 1.48 mg VS/g media) and one of the antibiotic-fed columns (1.55 mg VS/g media), with more biofilm VS in the second antibiotic fed column (1.91 mg VS/g media). Similar

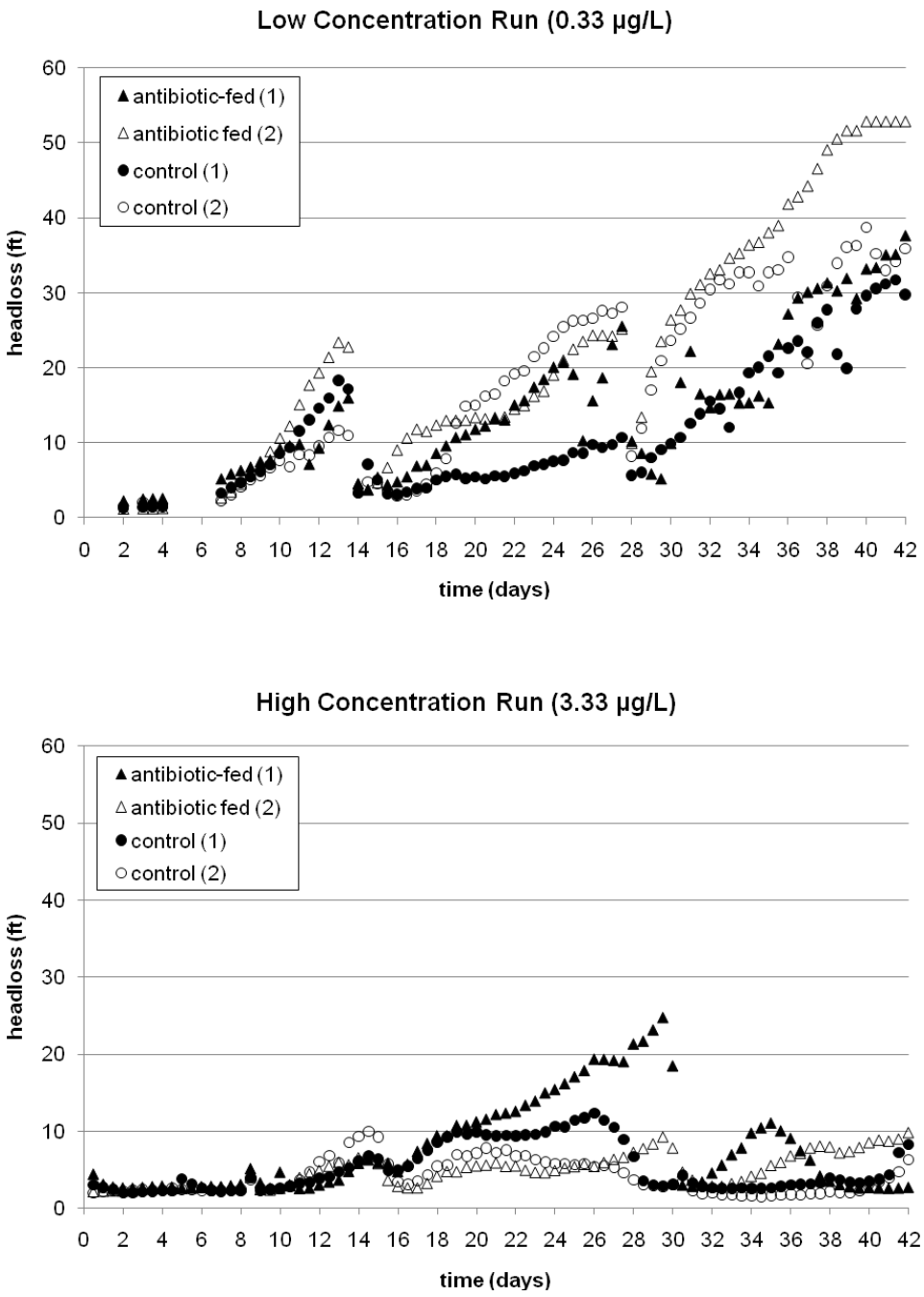

Figure 4.2. Filter column headloss data for bench-scale SSF experiments.

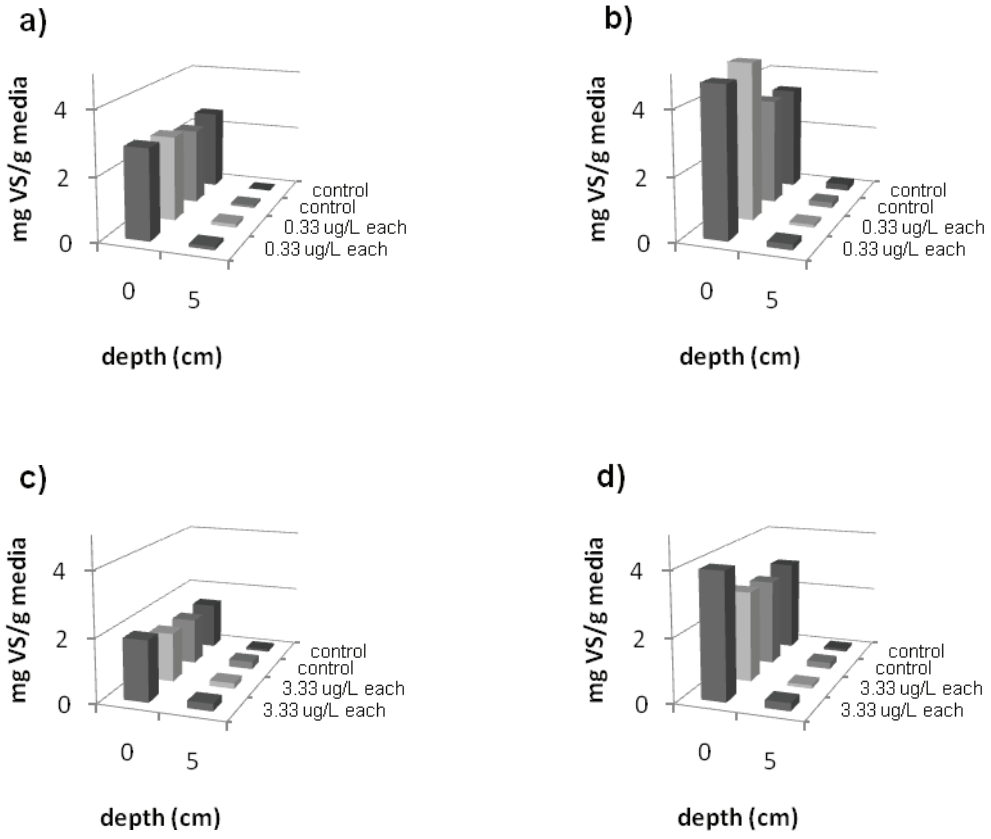

Figure 4.3. Biomass (as VS) in sand from filter columns. Plots shown: a) 0.33 µg/L (each antibiotic) and controls at 2 weeks operation, b) 0.33 µg/L (each antibiotic) and controls at 4 weeks operation, c) 3.33 µg/L (each antibiotic) and control at 2 weeks operation, and d) 3.33 µg/L(each antibiotic) and control at 4 weeks operation. VS determined for subsequent filter depths (*i.e.*, 10 and 15 cm) were negligible (< 0.10 mg VS/g media) and are not shown.

results were observed at four weeks. For all columns, regardless of feed condition, the amount of VS present at 5, 10, and 15 cm depths was approximately ten times less than VS quantities at zero depth.

4.3.2 Antibiotic Breakthrough

Antibiotic breakthrough in filter columns was assessed by considering the filtrate antibiotic concentration (relative to the feed concentration, C_0) as a function of filter run-time (Figure 4.4). Rapid breakthrough of SMX and ERY was observed, with considerable variability in ERY filtrate concentrations during earlier stages (*i.e.*, first 12 days) for the high feed condition. CIP breakthrough occurred at approximately 10 days of operation for the high feed condition and at 40 days for the low feed condition. Replicate columns for all conditions exhibited similar results.

The retardation/breakthrough model compares reasonably well with the experimental results. Predicted breakthrough times for ERY(<1.4 days) and SMX (<1.0 day) were very short because of the relatively low K_{oc} values for these compounds. Conversely, the predicted breakthrough time for CIP, with a much greater K_{oc} value was ~21 days. The predicted breakthrough times are especially sensitive to the variations in schmutzdecke f_{oc} and biofilm presence, which could explain differences between modeled and experimentally-determined breakthrough times for CIP.

4.3.3 Antibiotic Sorption to Schmutzdecke

From the batch tests, the K_{oc} values for CIP obtained for non-sterilized and sterilized schmutzdecke material were 98,000 and 210,000 L/kg, respectively (Figure 4.5). There was no sorption of either SMX or ERY to schmutzdecke (data not shown).

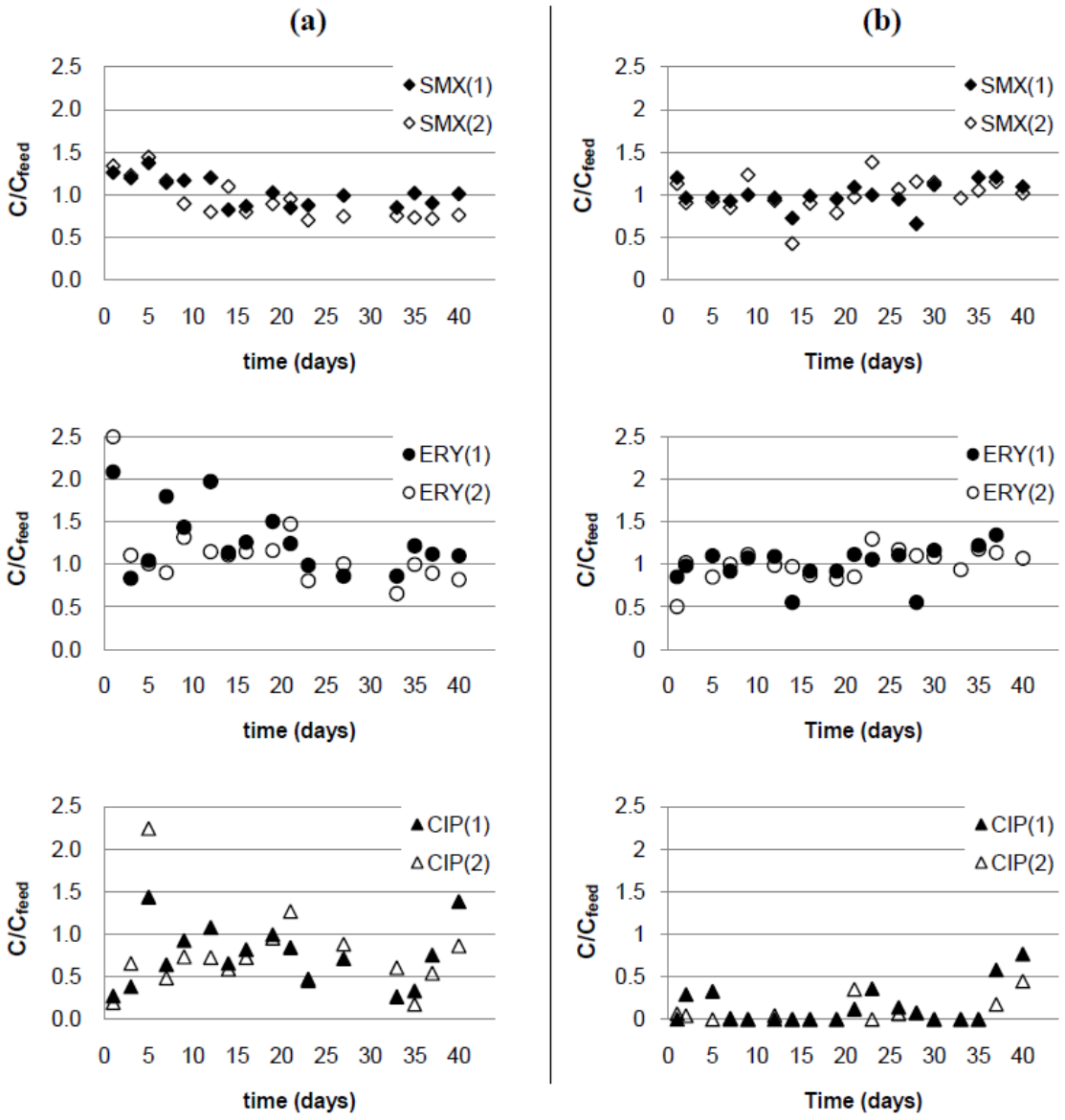

Figure 4.4. Filtration antibiotic concentration normalized to feed concentration (C/C_{feed}) versus time for bench-scale SSF. Plots for the following conditions are provided (a) 3.33 µg/L (each antibiotic), and (b) 0.33 µg/L (each antibiotic). Replicate columns are differentiated by open and closed symbols on each plot.

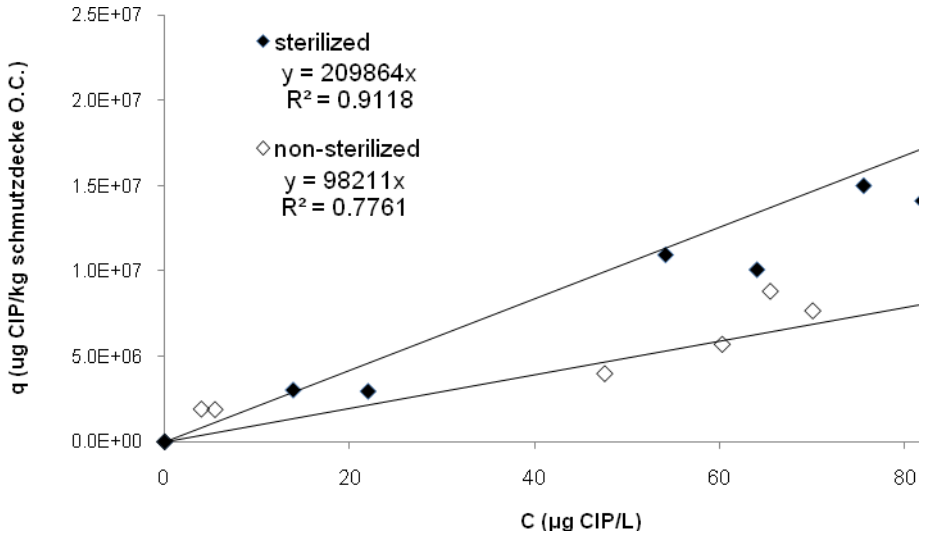

Figure 4.5. Sorption of CIP to schmutzdecke material. Isotherm data presented for sterilized (K_{oc} = 210,000 L/kg) and non-sterilized (K_{oc} = 98,000 L/kg) schmutzdecke material. Regression of data was forced to intercept at the origin.

4.3.4 Community Structure

Based on non-metric multidimensional scaling (nMDS) analysis of biofilm bacteria ARISA data (Figure 4.6), the bacteria community structure of antibiotic fed columns does not appear different than control columns. The community structure was dynamic, but stabilized at four weeks of operation (*i.e.*, no apparent difference between nMDS results at 4 and 6 weeks) for all zero-depth samples.

Tables with all fragment length versus % area data are provided in Appendices H and I. In general, the community compositions in the columns were similar, as numerous fragment lengths (*e.g.*, 453, 461, 468, 625, 629, 637, 673, and 690 bp) were present in all of the columns. There were, however, some fragment lengths that were present in the control and low antibiotic columns but not present in samples from the high antibiotic

feed condition (*e.g.*, fragment lengths 714 and 717 bp). These subtle differences in community composition suggest inhibition of some species at the higher antibiotic concentrations.

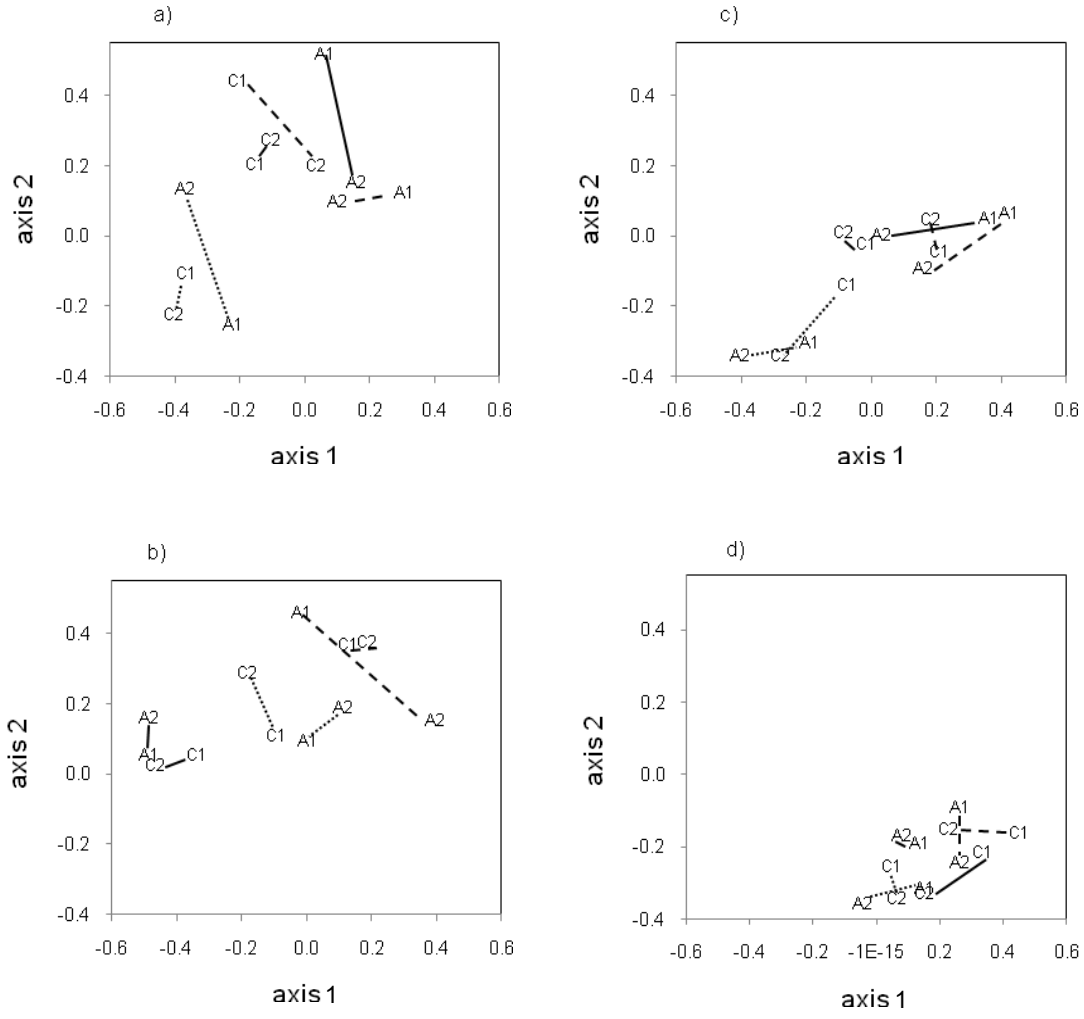

Figure 4.6. non-metric multidimensional scaling plots (nMDS) of biofilm bacteria ARISA results. Results for runs with: a) high antibiotic concentration (3.33 µg/L) feed condition at zero depth, b) high antibiotic concentration (3.33 µg/L) feed condition at 5-cm depth, c) low antibiotic concentration (0.33 µg/L) feed condition at zero depth, and d) low antibiotic concentration (0.33 µg/L) feed condition at 5-cm depth. Samples from replicate columns exposed to antibiotics are denoted A1 and A2, and control replicate columns samples are denoted C1 and C2. Connecting lines link replicate samples and represent the time of sample collection: 2 weeks (·····), 4 weeks (———), and 6 weeks (- - - - -).

4.4 Discussion

4.4.1 Biomass Accumulation and Headloss Development

The zero depth biomass values observed in this work (1.55 to 5.31 mg VS/g media) are consistent with those of Eighmy *et al*. (1992) and Yordanov *et al*. (1996) who reported ~2.5 mg protein/g dry media mass for surface schmutzdecke (*i.e*., zero depth). Eighmy *et al*. (1992) and Yordanov *et al*. (1996) also observed a significant decrease in biomass with increasing depth.

The reported headloss at the beginning of the experiments (*i.e*. "clean" filter without biomass present) was approximately 0.90 feet for all columns; these values are consistent with headloss values (0.75 ft) predicted by the Carman-Kozeny equation (assuming a porosity of 0.4 and sphericity of 0.75). The increasing headloss as biomass accumulated over the duration of experiments is consistent with other SSF experiments (Duncan, 1988; Eighmy *et al.*, 1994). With biofilm development the filter bed porosity decreases, requiring greater headloss to maintain constant filtration rates. Measured biomass quantities in the top portion of the filter column (through four weeks of operation) ranged from 1.47 to 5.31 mg VS/g media. These biomass values were used to compute the volume of biofilm occupying the pore space so that a porosity reduction could be estimated. Biofilm densities ranging from 5 to 130 kg VS/m^3 were reported by Characklis and Marshall (1990) for various biofilms. Using 5 mg VS/g media and assuming a biofilm density of 30 kg VS/m^3, the estimated porosity is 0.08; yielding a headloss of 47 ft. With a biofilm density of 50 kg VS/m^3 and 5 mg VS/g media, the estimate porosity is 0.19 and the headloss is 3 feet. These estimated headloss values were consistent with

observed range of headloss values for the mature filter column (*i.e.*, after 2 weeks of operation).

4.4.2 Antibiotic Sorption and Attenuation

Because antibiotic feed commenced immediately upon system inoculation, these experiments represent the start-up period of SSF operation. Rapid beakthrough of SMX and ERY was expected given low antibiotic-biofilm K_{oc} values (<6,000 L/kg) for these compounds (Chapter 3). The difference in CIP breakthough (~12 days for the high feed condition and ~40 days for the low feed condition is partially explained by greater amounts of biomass in the low antibiotic-feed condition columns, but also linked to the difference in concentration (*i.e.*, 0.33 vs. 3.33 μg/L) and biofilm capacity for antibiotic. Furthermore, use of the K_{oc} value for biofilm as sorbent (92,000 L/kg) to predict CIP breakthrough time for a SSF was reasonable as the K_{oc} values determined for CIP with schmutzdecke material as sorbent (98,000 L/kg and 210,000 L/kg**)** were similar. A reported K_{oc} value for sorption of CIP to soil was slightly lower (61,000 L/kg; Nowara, *et al*., 1997).

There was no evidence of biodegradation of SMX or ERY in the columns, as the antibiotics rapidly broke through and $C/C_0 \approx 1$ for the duration of the experiment. The results for CIP are not as clear given the temporary decline in normalized effluent concentrations between 28 and 40 days of operation, This decline in normalized effluent concentration corresponds with a sampling date (*i.e*. 28 days). It is possible that the disruption and removal of a small amount of media either resulted in CIP release or a

temporary decrease in column sorption capacity. Furthermore, in batch experiments with MDC schmutzdecke, there was less uptake of CIP by non-sterilized material than by sterilized material. The results of previous studies regarding biodegradation of the antibiotics used in this research are conflicting. ERY was slowly biodegraded ($t_{1/2}$ of 11.5 days) in aerobic soil and not biodegraded in wastewater treatment plant effluent in 40-day closed-bottle tests (Gavalchin and Katz, 1994; Alexy *et al*., 2004). Although SMX was not biodegraded in surface water or by activated sludge bacteria under aerobic conditions (Gavalchin and Katz, 1994; Al-Ahmad *et al*., 1999), biodegradation (half-lives > 1 day) was observed for activated sludge and river sediment (Radke *et al*., 2009; Li and Zhang, 2010), with the extent of SMX biodegradation greater under oxic conditions compared to anoxic conditions (Suarez *et al*., 2010). For CIP, biodegradation by activated sludge bacteria (half-life of $\geq$2 days) was reported (Rabolle and Splid, 2000; Halling-Sorensen *et al*., 2000), while others concluded that CIP is not biodegradable in by wastewater bacteria (Al-Ahmad *et al*., 1999; Wu *et al*., 2009; Li and Zhang, 2010).

4.4.3 SRBF System Modeling

With pertinent system information and reported partitioning coefficients for antibiotic sorption to media organic carbon, antibiotic retardation and breakthrough times in SSF, RBF, and rapid gravity (bio)filtration (RGBF) systems were estimated (Table 4.1). Additonal model parameter data is provided in Appendix J. Two antibiotics not used in the experiments performed in this research (oxytetracycline and efrotomycin) were included for comparison.

Rapid breakthough of antibiotics is expected for RGBF systems. Furthermore, breakthrough of even the most sorptive antibiotic (CIP) is expected within one month for SSF systems; potentially problematic since these systems require lengthy maturation periods (35 to 280 days) prior to use for water treatment (Cleasby and Haarhof, 1991). These results are contrasted by the modeling results of Rooklidge *et al*., (2005), who predicted 3-log removal of macrolide and quinolone antibiotics in slow sand filters after 60-days of operation, but are complemented by reported removals for low ng/L concentrations of SMX (26%) and ERY (no removal) in a SSF system treating secondary effluent of a municipal wastewater treatment plant (Nakada *et al*., 2007). Antibiotic breakthrough times should increase in uncovered SSF sytems where photosynthetic biomass in the schmutzdecke increases the sorption capacity (Campos *et al*., 2002).

Table 4.1. Antibiotic Retardation and Breakthrough in Biofiltration Systems.

Antibiotic K_{oc}, L/kg	**SSF**		**RBF**		**RGBF**	
	R^A	t_b (days)^B	R^A	t_b (days)^B	R^A	t_b (days)^B
CIP 92,000	2341, 56	**49.2** (24.4, 24.8)	3181, 81	**468.0** (132.5, 335.5)	147, 60	**0.55** (0.31,0.26)
ERY 6,000	157, 5	**3.68** (1.64, 2.04)	213, 6	**35.1** (8.9,26.2)	11, 5	**<0.1**
SMX 4,000	105, 4	**2.59** (1.09,1.50)	142, 5	**15.4** (5.9,9.5)	7.5, 4	**<0.1**
Oxytetracycline 50,000	1301, 34	**16.68** (8.14,8.54)	1768, 45	**118.1** (51.6,66.5)	82, 34	**0.31** (0.17,0.14)
Efrotomycin 1,000	4, 1	**0.75** (0.17,0.58)	5, 1	**4.44** (1.07,3.37)	1, 1	**<0.1**

[A]Retardation factors (R) are presented first for the biological zone (i.e., schmutzdecke or colmation zaone) followed by the abiotic filtration zone (i.e., filter sand or aquifer material).
[B]Breakthough times (tb)are presented for the entire system in bold, with parenthetical numbers representing tb for the biological zone followed by the abiotic filtration zone.

Among the SRBF processes considered, antibiotic retardation is expected to be greatest in RBF systems due to the presence in dense colmation zone material. For the assumed conditions, CIP is expected to break through in 468 days. It is noted that the composition and configuration of RBF systems is site specific and temporally variable. Antibiotic breakthough in RBF would depend on site specific conditions, such as water velocity and travel distance. Furthermore, loss of colmation zone material via scour and other factors could significantly affect the breakthrough times. Heberer *et al.* (2008) reported removals of ERY (90%) and SMX (52 and 99% under oxic and anoxic conditions, respectively) for a full-scale bank filtration system (~1 month travel time). Similar results were reported for SMX by Grünheid *et al*. (2005).

4.4.4 Community Structure

Major microbial community shifts were not evident when comparing control columns with filter columns receiving antibiotics. It is diffcult to directly compare recent work showing adverse impacts of antibiotic mixtures on bacteria in environmental settings (*i.e.* Yang *et al*., 2008; Ghosh *et al*., 2009) because the conditions were different from those employed in this work. For example, Yang *et al*. (2008) exposed a single microbial species to a larger number (5) of antibiotics (each at 0.1 μg/L) while Ghosh *et al*. (2009) used much higher antibiotic concentrations (>50 μg/L). The results presented in Chapter 2 indicate that low μg/L concentrations of SMX, CIP, and ERY impact the community structure of biofilm bacteria in continuous-feed rotating bioreactors.

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The minimum inhibitory concentrations (MIC) of SMX, ERY, and CIP are 3 to 15,000 times higher than the highest concentration (3.33 μg/L) used in this study. Acting as an anolog to *p*-aminobenzoic acid, SMX blocks sysnthesis of folic acid in both gram positive (MIC of 16 mg/L for *Escherichia coli*) and gram negative (MIC of 32 mg/L for *Staphylococcus aureus*) bacteria (Brock and Madigan, 1991; Lorian, 1996). CIP is a broad spectrum antibiotic that inhibits the action of the enzymes topoisomerase IV and DNA gyrase, with MICs of 0.25 mg/L for *S. aureus* and 0.01 mg/L for *E. coli* (Lorian, 1996; Pan *et al*., 1996). ERY inhibits protein synthesis in gram positive bacteria (0.25 mg/L for *S. aureus*) by binding to 23S rRNA and blocking entrance to the 50S subunit of the ribosome (Lorian, 1996; Tenson *et al*., 2003). Folic acid produced by other biofilm bacteria unaffected by SMX, however, might be available for use by bacteria whose folic acid production is inhibited by SMX.

Biologically-active filters are dominated by aerobic gram-positive eubacteria (Fonseca *et al*., 2001); species likely least affected by the combined action of ERY and CIP (with SMX action neutralized). The robust community structure of the biofilm may be attributed to the presence of resistant bacteria, but no attempt was made to isolate and study the antibiotic resistance of individual strains. Since no effect on the presence of indivual fragment lengths was evident at low concentration (0.33 μg/L) feed conditions, the presence (or absence) of individual fragment lengths (*e.g.*, 714 and 717 bp) at high concentrations (3.33 μg/L) might be due to antibiotic activity. Effects could be due to antibiotic action against susceptible species (*e.g.*, gram negative bacteria), or susceptibility of these bacteria to (normally) sub-inhibitory concentrations of highly

potent CIP. Similar impacts on individual species were discussed in Chapter 2. The loss of individual organisms is of concern if the organisms are essential for the transformation of specific compounds (*e.g.*, nitrate, microcontaminants) not degraded by other community bacteria.

4.5 Conclusions

Regardless of feed concentration, SMX and ERY exhibited rapid breakthrough during start-up conditions in in bench-scale SSF columns. CIP breakthrough was slower for low concentration feed conditions compared to high concentration feed conditions. The presence of antibiotics in bench-scale SSF systems did not adversely effect biomass development or bacteria community structure. The results of modeling indicate that the breakthrough of highly sorptive antibiotics (*e.g.*, CIP) in full-scale SSF occurs during (or closely following) maturation stages, but these systems typically receive higher quality source water less influenced by anthropogenic activities linked to antibiotic use and application. The results of modeling indicate that antibiotic breakthrough times in RBF systems are approximately ten times that of SSF, but will vary depending and site and system conditions. Ultimately, other proven treatment processes (*e.g.*, reverse osmosis/nanofiltration, granular activated carbon, and advanced oxidation processes) should be employed by systems intent on removing antibiotics from finished water supplies.

4.6 References

Adams, C., Wang, Y., Loftin, K., Meyer, M. (2002) Removal of antibiotics from surface and distilled water in conventional water treatment processes, Journal of Environmental Engineering 128(3), 253-260.

Al-Ahmad, A., Daschner, F.D., Kummerer, K. (1999) Biodegradability of Cefotiam, Ciprofloxacin, Meropenem, Penicillin G, and Sulfamethoxazole and inhibition of waste water bacteria. Archive of Environmental Contamination and Toxicology 37(2), 158-163.

Alexy, R., Kümpel, T., Kümmerer, K. 2004. Assessment of degradation of 18 antibiotics in the Closed Bottle Test. Chemosphere 57(6), 505-512.

Benotti, M.J., Trenholm, R.A., Vanderford, B.J., Holady, J.C., Stafford, B.D., Snyder, S.A . (2009) Pharmaceuticals and endocrine disrupting compounds in US drinking water. Environmental Science &Technology 43(9), 597-603.

Brock, T.D.; Madigan, M.T. (1991) *Biology of Microorganisms, 6th Ed*. Prentice-Hall, Englewood Cliffs, NJ, USA.

Campos, L.C., Su M.F.J., Graham N.J.D.; Smith S.R.(2002) Biomass development in slow sand filters. Water Research 36(18):4543-4551.

Chamberlain, E., Adams, C. (2006) Oxidation of sulfonamides, macrolides, and carbadox with free chlorine and monochloramine. Water Research 40(13), 2517-2526.

Characklis, W.G., Marshal, K.C. (1990) *Biofilms*, Wiley-Interscience, New York, 1990

Cleasby, J.L., Haarhoff, J. (1991) Biological and Physical Mechanisms in Slow Sand Filtration. *In* Slow Sand Filtration. Edited by G. Logsdon. ASCE Reston, VA.

Collins, M.R., Eighmy, T.T., Fenstermacher, J.M., Spanos, S.K (1989) Modifications to the Slow Sand Filtration Process For Improved Removals of Trihalomethane Precursors. AWWARF, Denver, CO.

Dodd, M.C., Huang, C-H. (2004) Transformation of the antibacterial agent sulfamethoxazole in reactions with chlorine: kinetics, mechanisms, and pathways. Environmental Science & Technology 38(21), 5607-5615.

Drillia, P., Stamatelatou, K., Lyberatos, G. (2005) Fate and mobility of pharmaceuticals in solid matrices. Chemosphere 60, 1034–1044.

Duncan, A. (1988) The ecology of slow sand filters.In proceedings: Slow sand filtration: recent development in water treatment technology.Chichester, UK.

Eighmy, T.T., Collins, M.R., Spanos, S.K., Fenstermacher, J (1992) Microbial populations, activities and carbon metabolism in slow sand filters. Water Research 26(10), 1319-1328.

Eighmy, T.T., Spanos, S.K., Royce, J., Collins, M.R., Malley, J.P. (1994) Microbial activity in slow sand filters.In proceedings: Slow sand filtration. AWWA, Denver, CO

Flaherty, C.M.; Dodson, S.I (2005). Effects of pharmaceuticals on *Daphnia* survival, growth, and reproduction Chemosphere 61(2), 200-207.

Fonseca, A.C.; Summers, R.S.; Hernandez, M.T. (2001) Comparative measurements of microbial activity in drinking water biofilters. Water Research 36(16), 3817-3824.

Gavalchin, J., Katz, S.E. (1994) The persistence of fecal-borne antibiotics in soil. Journal of the Association of Official Analytical Communities International 77(2), 481-485.

Ghosh, G.C., Okuda, T., Yamashita, N. (2009) Occurrence and elimination of antibiotics at four sewage treatment plants in Japan and their effects on bacterial ammonia oxidation. Water Science and Technology 59(4), 779-786.

Giger, W.; Alder, A.C.; Golet, E.M.; Kohler, H.P.E.; McArdell, C.S., Molnar, E.; Siegrist, H.; Suter, M.J.F. (2003). Occurrence and fate of antibiotics as trace contaminants in wastewaters, sewage sludges, and surface waters. Chimia 57(9), 485-491.

Grünheid, S., Amy, G., Jekel, M. (2005) Removal of bulk dissolved organic carbon (DOC) and trace organic compounds by bank filtration and artificial recharge. *Water Res.* 39(14), 3219–3228.

Halling-Sorensen, B., Holten Lutzhoft, H.-C., Anderson, H.R., Ingerslev, F. (2000) Environmental risk assessment of antibiotics: comparison of mecillinam, trimethoprim and ciprofloxacin. Journal of Antimicrobial Chemotherapy 46(suppl. 1): 53-58.

Heberer, T., Massmann, G., Frank, B., Taute, T., Dünnbier, U. (2008) Behavior and redox sensitivity of antimicrobial residues during bank filtration. *Chemosphere* **73**, 451–460.

Hiscock, K.M., Grischek, T. (2002) Attenuation of groundwater pollution by bank filtration. Journal of Hydrology 266(3-4), 139-144.

Huber, M. M., Korhonen, S., Ternes, T. A., von Gunten, U. (2005) Oxidation of pharmaceuticals during water treatment with chlorine dioxide. Water Research 39(15), 3607-3617.

Kawamura S. 1991. *Integrated Design of Water Treatment Facilities*. John Wiley and Sons, New York, NY, USA.

Kim, S.D.; Cho, J.;Kim, I.S.; Vanderford, B.J.; Snyder, S.A. (2007) Occurrence and removal of pharmaceuticals and endocrine disruptors in South Korean surface, drinking, and waste water Water Research 41(5), 1013-1021.

Knapp, C.W., Engemann, C.A., Hanson, M.L., Keen, P.L., Hall, K.J., Graham, D.W. (2008) Indirect evidence of transposon-mediated selection of antibiotic resistance genes in aquatic systems at low-level oxytetracycline exposures. Environmental. Science and Technology 42(14), 5348–5353.

Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., Buxton, H.T. (2002). Pharmaceuticals, hormones, and other organic wastewater constituents in US streams, 1999-2000: a national reconnaissance. Environmental Science and Technology 36(6), 1202-1211.

Kuehn W, Mueller U. (2000) Riverbank filtration: an overview. Journal AWWA 92(12), 60-69.

Li, B., Zhang, T. (2010) Biodegradation and Adsorption of Antibiotics in the Activated Sludge Process. Environmental Science and Technology. 44(9), 3468-3473.

Lorian V. 1996. *Antibiotics in Laboratory Medicine*. Lippincott Williams & Wilkins, Baltimore, MD, USA.

Muñoz-Aguayo, J., Lang, K.S., LaPara, T.M., González, G., Singer, R.S. (2007) Evaluating the effects of chlortetracycline on the proliferation of antibiotic-resistant bacteria in a simulated river water ecosystem. Applied Environmental Microbiology 73(17), 5421-5425.

Nakada, H.,Shinohara, H., Murata, A., Kiri, K., Managaki, S., Sato, N., Takada, H. (2007) Removal of selected pharmaceuticals and personal care products (PPCPs) and endocrine-disrupting chemicals (EDCs) during sand filtration and ozonation at a municipal sewage treatment plant Water Research 41(19), 373-4382.

Ngheim, L.D., Schäfer, A.I., Elimelech, M. (2005) Pharmaceutical retention mechanisms by nanofiltration membranes. Environmental Science & Technology 39(19), 7698-7705.

Nowara, A., Burhenne, J., Spiteller, M. (1997) Binding of fluoroquinolone carboxylic acid derivatives to clay minerals. Journal of Agricultural and Food Chemistry 45(4), 1459–1463.

Pan, X.S., Ambler, J., Mehtar, S., Fisher, L.M. (1996) Involvement of topoisomerase IV and DNA gyrase as ciprofloxacin targets in Streptococcus pneumonia. Antimicrobial Agents and Chemotherapy. 40(10), 2321-2316.

Rabolle, M., Spliid, N.H. (2000) Sorption and mobility of metronidazole, olaquindox, oxytetracycline and tylosin in soil. Chemosphere 40(7), 715-722.

Radke, M., Lauwigi, C., Heinkele, G., Murdter, T.E., Letzel, M. (2009) Fate of the antibiotic sulfamethoxazole and its two major human metabolites in a mater sediment test. Environmental Science and Technology 43 (9), 3135-3141.

Rooklidge, S.J., Burns, E.R., Bolte, J.P. (2005) Modeling Antimicrobial contaminant removal in slow sand filtration. Water Research 39(2-3), 331-339.

Snyder, S.A., Adham, S., Redding, A.M., Cannon, F.S., DeCarolis, J., Oppenheimer, J., Yoon, Y. (2007) Role of membranes and activated carbon in the removal of endocrine disruptors and pharmaceuticals. Desalination 202(1-3), 156-181.

Suarez, S., Lema, J.M., Omil, F. (2010) Removal of Pharmaceutical and Personal Care Products (PPCPs) under nitrifying and denitrifying conditions Water Research 44(10), 3214-3224.

Tenson, T., Lovmar, M., Ehrenberg, M. (2003) The mechanism of action of macrolides, linosamides, and streptogramin B reveals the mascent peptide exit path in the ribosome. Journal of Molecular Biology. 330(5), 1005-1014.

Weber-Shirk, M., Dick, R.I. (1997) Physical-chemical mechanisms in slow sand filters. Journal AWWA, 89(1), 87-100.

Westerhoff, P., Yoon, Y., Snyder, S., Wert, E. (2005) Fate of endocrine-disruptor, pharmaceutical, and personal care product chemicals during simulated drinking water treatment processes. Environmental Science & Technology, 39(17), 6649-6663.

Wiegel, S., Aulinger, A., Brockmeyer, R., Harms, H., Löffler, J., Reincke, H., Schmidt, R., Stachel, B., von Tümpling, W., Wanke, A. (2004) Pharmaceuticals in the river Elbe and its tributaries. Chemosphere 57(2), 107-126.

Wu, C., Spongberg, A.L., Witter, J.D. (2009) Sorption and biodegradation of selected antibiotics in biosolids. Journal of Environmental Science and Health Part A 44(5) 454-461.

Yang, L,-H., Ying, G,-G., Su, H.C., Stauber, J.L., Adams, M.S., Binet, M.T. (2008) Growth-inhibiting effects of 12 antibacterial agents and their mixtures on the freshwater microalga *Pseudokirchneriella subcapitata*. Environmental Toxicology and Chemistry 27(5), 1201–1208.

Yordanov, R.V., Lamb, A.J., Melvin, M.A.L., Littlehohn, J. (1996) Biomass characteristics of slow sand filters receiving ozonated water. In proceedings: Advances in slow sand and alternative biological filtration. UK: Wiley

Chapter 5. Conclusions

The investigation and elucidation antibiotic interactions in slow-rate biofiltration processes have yielded important information regarding the fate and impact of antibiotics in systems that rely on biofilm bacteria. The results presented in Chapter 2 provide valuable information for understanding and modeling antibiotic fate in biofilm. In particular, this will benefit those interested in predicting the sorptive fate of antibiotics in systems that rely on biofilm (*i.e.*, biofiltration) and bacterial EPS (*i.e.*, bioflocculation); shedding light on key system parameters that dictate how antibiotics interact with biofilm and EPS. The results of Chapter 3 have improved understanding of how antibiotics might affect the structure and function of biofilm bacteria communities. Leveraging the findings of earlier chapters with complementary work, the results of Chapter 4 have advanced the understanding of antibiotic attenuation and effects in SRBF processes. This will benefit those interested in modeling the transport of antibiotics in saturated porous media, as well as those planning and designing water treatment systems (that include biofiltration) for antibiotics removal. The results of this research are broadly applicable to a variety of engineered and natural water treatment processes for which the biofilm bacteria are critical to system performance.

5.1 Antibiotic Sorption to Biofilm

The structural properties of an antibiotic control the rate and extent of its sorption to biofilm. The extent (K_{oc}) and rate (k) of sorption for CIP to biofilm was greater than found for ERY or SMX. That antibiotic partitioning to biofilm as described by K did not correlate with the antibiotic octanol-water partitioning coefficients (K_{ow}) suggests that

hydrophobic interactions are unimportant for sorption of these relatively hydrophilic compounds to biofilm. Antibiotic speciation (*i.e.*, degree of ionization) partially explains the extent of sorption to biofilm, but similarly ionized antibiotics (*i.e.*, CIP^+ and ERY^+) displayed vastly disparate levels of sorption; possibly due to molecular size differences (*i.e.*, ERY>CIP) and mass transfer rates. SMX is anionic and fully ionized at circum-neutral pH, while CIP and ERY are predominantly cationic with potential to deionize with increasing pH. The relative extent of sorption to biofilm (CIP>>ERY>SMX) is expected given the different degrees of antibiotic ionization. Because antibiotic speciation and biofilm properties are affected by changes in pH, changes in system pH are expected to affect the retention (or desorption) of antibiotics sorbed to biofilm.

The results of this work are significant to those concerned with modeling the antibiotic fate in biologically-active filtration systems used for drinking water production, including slow sand filtration, riverbank filtration, and rapid (bio)filtration. The results are also valuable for predicting the fate of antibiotics in other systems where biofilms (and EPS) are present including: drinking water distribution networks, aquifer recharge installations, soil-aquifer treatment applications, wastewater fixed-film bioreactors, activated sludge proceses, and septic system leach fields. Finally, the results of this work inform the selection or development of compounds intended for inactivation of bacterial biofilms.

5.2 Effect of Antibiotics on the structure and function of biofilm bacteria

The results of this work indicate that mixtures of antibiotics at concentrations relevant to surface water treatment applications (*i.e.*, sub-μg/L) are unlikely to adversely impact the

overall performance of biofiltration systems in terms of readily biodegradable substrates. Should concentrations of low MIC antibiotics (*i.e.*, CIP) approach the low μg/L concentration range, there is some concern that negative effects may be manifest for drinking water biofiltration systems, such as shifts in bacterial community structure and increased accumulation of biomass. Microbial community shifts might have negative implications if specific compounds (targeted for removal) are biodegraded by species that are sensitive to antibiotics, or if antibiotic resistant bacteria are proliferated during the biofiltration process. If linked to increased headloss development, conditions that induce higher biomass accumulation (low μg/L CIP) could prove problematic for biofilter performance and operation.

The implications of this work may be more profound where higher concentrations of antibiotics are likely present, such as wastewater treatment fixed film and biofiltration processes. Continued diligence with controlled antibiotic use and disposal is warranted to prevent further increases of antibiotic concentrations in aquatic systems.

5.3 Bioattenuation and Effect of Antibiotics in Slow-rate Biofiltration Processes. Antibiotic removal in SRBF processes is system, compound, and run-time specific. CIP removal in bench-scale SRBF filter columns is greater than that of ERY and SMX; expected results based on antibiotic-biofilm K_{oc} values, used also to adequately predict antibiotic breakthrough in filter columns. Antibiotic biodegradation was not shown for any antibiotic in bench-scale SRBF filter columns. Results from a one dimensional advection model assuming linear sorption for full-scale SSF, RBF, and RGBF systems

indicated that: no antibiotic removal can be expected for RGBF systems; even the most sorptive antibiotics (*e.g.*, CIP) will saturate media (and reach breakthrough) during lengthy maturation periods used for SSF systems; some antibiotics will be removed in RBF systems, due to sorption processes and possibly biodegradation (especially under anoxic conditions). For the studied conditions, non-sorbed antibiotics that interact with bacteria do not broadly affect the community structure, but may impact a few individual species.

Ultimately, other proven treatment processes (*e.g.*, reverse osmosis/nanofiltration, and advanced oxidation processes) should be employed by systems intent on removing antibiotics from finished water supplies. The results of this work are valuable for those concerned with the removal of antibiotics in biofiltration systems used for drinking water and wastewater treatment, and largely alleviate concerns associated with the adverse impacts of antibiotic mixtures (at environmentally-relevant concentrations) on biofilm bacteria of biofiltration processes.

5.4 Recommendations for Future Research

5.4.1 Sorption/Desorption Mechanisms

This sorption experiments were conducted using a mineral medium with a fixed pH under oxic conditions for a multi-species biofilm. More research is needed to elucidate the mechanisms operative in the sorption of antibiotics (and other acidic pharmaceuticals) to biofilm. Specifically, the EPS sorption sites that are relevant for acidic pharmaceuticals should be identified. Although typically negatively charged above pH 7, biofilm EPS

contains ionic functional groups with exchange potential for both cations and anions (Liao *et al*., 2001), and apolar functional groups (*e.g.*, aromatic) that might sorb apolar organic compounds (Flemming *et al*., 1996). And its hydrophilic nature (Bryers, 2000) may also provide for nonionic interactions. A mechanistic study would consider the importance of pH on antibiotic-biofilm sorption, retention, and desorption, and should account for a range of pH values typical for varied surface water treatment applications and relevant for numerous acidic pharmaceuticals that are detected in surface waters.

5.4.2 Effect of Antibiotics on Bacteria that Degrade or Transform Contaminants. The results of this work showed shifts in microbial community structure at low μg/L concentrations. Shifts in microbial community structure might negatively impact biodegradation of specific compounds if those compounds are only degraded by a single or few species that are also sensitive to the antibiotics. More work could be done to investigate the affects of additional antibiotics and combinations (not tested here) on the structure and function of aerobic heterotrophs utilizing biodegradable natural organic matter, as well as other bacteria of relevance in biofiltration systems operated under conditions that favor nitrification or denitrification.

5.4.3 Biodegradation of Antibiotics under Environmentally-relevant Conditions

No biodegradation was apparent in results of this work, and contrasting results of antibiotic biodegradation have been reported by others (Al-Ahmad *et al*., 1999; Alexy *et al*., 2004; Dantas *et al*., 2008; Wu *et al*., 2009; Li and Zhang, 2010), with no research specifically focused on biodegradation of antibiotics at or below low μg/L concentrations.

Future research on antibiotic biodegradation is warranted and should consider the following factors: the presence and absence of a primary substrate, acclimation, source of bacteria, oxic and anoxic conditions, daughter compounds and their potency, and system temperature. Ultimately, antibiotics at concentrations typical for surface waters may be below minimum substrate (*i.e.* less than S_{min}) and not support biofilm bacteria growth and energy needs (Bouwer and McCarty, 1984).

5.5 References

Alexy, R., Kümpel, T., Kümmerer, K. 2004. Assessment of degradation of 18 antibiotics in the Closed Bottle Test. Chemosphere 57(6), 505-512.

Al-Ahmad, A., Daschner, F.D., Kummerer, K. (1999) Biodegradability of Cefotiam, Ciprofloxacin, Meropenem, Penicillin G, and Sulfamethoxazole and inhibition of waste water bacteria. Archive of Environmental Contamination and Toxicology 37(2), 158-163.

Bouwer, E.J., McCarty, P.L.(1984) Modeling of trace organics biotransformation in the subsurface. Groundwater 22(4), 433-440.

Bryers, J. *Biofilms II-Process Analysis and Applications*. (2000) Wiley, New York, NY.

Dantas, G., Sommer, M.O.A., Oluwasegun, R.D., Church, G. (2008) Bacteria Subsisting on Antibiotics, Science 320(5872), 100-103.

Flemming, H.-C., Schmitt, J., Marshall, K. C. (1996) In *Sorption properties of biofilms*. *Sediments Toxicology Substances*; Calmano, W., Foerstner, U., Eds.; Springer: Berlin, Germany, pp 115-157.

Li, B., Zhang, T. (2010) Biodegradation and Adsorption of Antibiotics in the Activated Sludge Process. Environmental Science and Technology. 44(9), 3468-3473.

Liao, B.Q., Allen, D.G., Droppo, I.G., Leppard, G.G., Liss, S.N. (2001) Surface Properties of sludge and their role in bioflocculation and settleability. Water Research 35(2), 339-350.

Wu, C., Spongberg, A.L., Witter, J.D. (2009) Sorption and biodegradation of selected antibiotics in biosolids. Journal of Environmental Science and Health Part A 44(5) 454-461.

Bibliography

Acar, J.F. (2000) Antibiotic synergy and antagonism. Medical Clinics of North America 84(6), 1391-1406.

Adams, C., Wang, Y., Loftin, K., Meyer, M. (2002) Removal of antibiotics from surface and distilled water in conventional water treatment processes, Journal of Environmental Engineering 128(3), 253-260.

Al-Ahmad, A., Daschner, F.D., Kummerer, K. (1999) Biodegradability of Ceftiam, Ciprofloxacin, Meropenem, Penicillin G, and Sulfamethooxazole and inhibition of waste water bacteria. Archive of Environmental Contamination and Toxicology 37(2), 158-163.

Alexy, R., Kümpel, T., Kümmerer, K. (2004) Assessment of degradation of 18 antibiotics in the Closed Bottle Test. Chemosphere 57(6), 505-512.

APHA. (1995) *Standard Methods for the Examination of Water and Wastewater*, 19th Ed. American Public Health Association, Washington, D.C.

Armstrong, J.L., Shigeno, D.S., Calomiris, J.J., Seidler, R.J. (1981) Antibiotic-resistant bacteria in drinking water. Applied Environmental Microbiology 42(2), 277-283.

Ash, R.J., Mauck, B., Morgan, M. (2002) Antibiotic resistance of gram negative bacteria in rivers, United States. Emerging Infectious Diseases 8(7), 713-716.

Bailey J. E. Ollis D. F. (1986) Biochemical Engineering Fundamentals. McGraw-Hill, Inc., New York, NY.

Baker, J.R., Mihelcic, J.R., Luehrs, D.C., Hickey, J.P. (1997) Evaluation of estimation methods for organic carbon normalized sorption coefficients. Water Environment Research 69(2), 136-145.

Batt, A.L., Bruce, I.B., Aga, D.S. (2006) Evaluating the vulnerability of surface waters to antibiotic contamination from varying wastewater treatment plant discharges. Environmental Pollution142(2), 295-302

Belden, J.B., Maul, J.D., Lydy, M.J. (2007) Partitioning and photodegradation of ciprofloxacin in aqueous systems in the presence of organic matter Chemosphere 66, 1390–1395.

Benotti, M.J., Trenholm R.A., Vanderford B.J., Holady J.C., Stafford B.D., Snyder S.A. (2009) Pharmaceuticals and endocrine disrupting compounds in US drinking water. Environmental Science &Technology 43(9), 597-603.

Berney,M., Hammes, F., Bosshard, F., Weilenmann, H.U. Egli, T. (2007) Assessment and interpretation of bacterial viability by using the LIVE/DEAD *Bac*Light Kit in combination with flow cytometry. Applied Environmental Microbiology **73,** 3283–3290.

Beveridge, T.J. Koval, S.F. (1981) Binding of metals to cell envelopes of Escherichia coli K-12. Applied Environmental Microbiology 42(2), 325-335.

Beveridge, T.J. (1984) Bioconversion of inorganic materials: mechanisms of the binding of metallic ions to bacterial walls and the possible impact on microbial ecology. In: Klug, M.J.; Reddy, C.A. (eds) Current perspectives in Microbial Ecology. American Society of Microbial Ecology, Washington, D.C. pp 601-607.

Bouwer, E.J., McCarty, P.L.(1984) Modeling of trace organics biotransformation in the subsurface. Groundwater 22(4), 433-440.

Bouwer, E.J. Crowe, P.B. (1988) Biological processes in drinking water treatment. Journal AWWA 80(9), 82-93.

Bouwer, E., Weiss, J., Ball, W., O'Melia, C., Arora, H. (1999) Water quality improvements during riverbank filtration at three Midwest utilities. Proc. International Riverbank Filtration Conference, Louisville, KY, USA.

Bouwer, E.J. (2003) Riverbank Filtration: The American Experience. In proc. 2nd International Riverbank Filtration Conference, Cincinnati, OH, USA.

Boxall, A.B.A., Kolpin, D.W., Halling-Sørensen, B. Tolls, J. (2003) Are veterinary medicines causing environmental risks? Environmental Science and Technology 37(15), 286A-294A.

Brock, T.D.; Madigan, M.T. (1991) *Biology of Microorganisms, 6th Ed*. Prentice-Hall, Englewood Cliffs, NJ, USA.

Bryers, J. *Biofilms II-Process Analysis and Applications*. (2000) Wiley, New York, NY.

Burhenne, J., Ludwig, M., Spiteller, M. (1997) Photolytic degradation of fluoroquinolone carboxylic acids in aqueous solution. 1. Primary photoproducts and half-lives. Environmental Science and Pollution Research 4(1), 10-15.

Calamari, D., Zuccato, E., Castigliono, S., Bagnati, R., Fanelli, R. (2003) Strategic survey of therapeutic drugs in the rivers Po and Lambro in northern Italy. Environmental Science and Technology 37(7), 1241-1248.

Campos, L.C., Su M.F.J., Graham N.J.D.; Smith S.R.(2002) Biomass development in slow sand filters. Water Research 36(18):4543-4551.

Carballa, M., Fink, G., Omil, F., Lema, J.M., Ternes, T. (2008) Determination of the solid-water distribution coefficient (K_d) for pharmaceuticals, estrogens, and musk fragrances in digested sludge. Water Research 42(1-2), 287-295.

Cardinale, M., Brusetti, L., Quatrinia, P., Borin, S., Puglia, A., Rizzi, A., Zanardini, E., Sorlini, C., Corselli, C. Daffonchio, D. (2004) Comparison of different primer sets for use in automated ribosomal intergenic spacer analysis of complex bacterial communities. Applied and Environmental Microbiology 70(10), 6147-6156.

Čermák, L., Kopecký, J., Novotná J., Omelka, M., Parkhomenko, N., Plháčková, K., Ságová-Marečková, M. (2008) Bacterial communities of two contrasting soils reacted differently to lincomycin treatment. Applied Soil Ecology 40, 348–358.

Černohorská, L., Voltava, M. (2008) Antibiotic Synergy against biofilm-forming *Pseudomonas aeruginosa*. Folia Microbiology 53(1), 57–60.

Chamberlain, E., Adams, C. (2006) Oxidation of sulfonamides, macrolides, and carbadox with free chlorine and monochloramine. Water Research 40(13), 2517-2526.

Characklis, W.G., Marshal, K.C. (1990) *Biofilms*, Wiley-Interscience, New York, 1990

Cleasby, J.L.and Haarhoff, J. (1991) Biological and Physical Mechanisms in Slow Sand Filtration. *In* Slow Sand Filtration. Edited by G. Logsdon. ASCE Reston, VA.

Collins, M.R., Eighmy, T.T., Fenstermacher, J.M., Spanos, S.K (1989) Modifications to the Slow Sand Filtration Process For Improved Removals of Trihalomethane Precursors. AWWARF, Denver, CO.

Congliang, Z., Yan, W., Fuan, W. (2007) Determination and Temperature Dependence of *n*-Octanol/Water Partition Coefficients for Seven Sulfonamides from (298.15 to 333.15) K. Bulletin of the Korean Chemical Society 28(7), 1183-1186.

Costanzo, S.D., Murby, J. Bates, J. (2005) Ecosystem response to antibiotics entering the aquatic environment. Marine Pollution Journal 51(1-4), 218-223.

Cottarel, G. Wierzbowski, J. (2007) Combination drugs, an emerging option for antibacterial therapy. Trends in Biotechnology 25(12), 547-555.

Dantas, G., Sommer, M.O.A., Oluwasegun, R.D., Church, G. (2008) Bacteria Subsisting on Antibiotics, Science 320(5872), 100-103

Davies, A.K., McKellar, J.F., Phillips, G.O.; Reid, A.G. (1979) Photochemical oxidation of tetracycline in aqueous solution. Journal of Chemical Society-Perkin Trans. II 1979(3), 369-375.

Dodd, M.C., Huang, C-H. (2004) Transformation of the antibacterial agent sulfamethoxazole in reactions with chlorine: kinetics, mechanisms, and pathways. Environmental Science & Technology 38(21), 5607-5615.

Drakapoulos, A.I. Ioannou, P.C. (1997) Spectrofluorimetric study of the acid-base equilibria and complexation behavior of the fluoroquinolone antibiotics ofloxacin, norfloxacin, ciprofloxacin and pefloxacin in aqueous solution. Analytical Chemistry Acta 354(1), 197-204.

Drillia, P., Dokianakis S.N., Fountoulakis M.S., Kornros M., Stamatelatou K. Lyberatos G. (2005) On the occasional biodegradation of pharmaceuticals in the activated sludge process: The example of the antibiotic sulfamethoxazole. Journal of Hazardous Materials 122(3), 259- 265.

Drillia, P., Stamatelatou, K., Lyberatos, G. (2005) Fate and mobility of pharmaceuticals in solid matrices. Chemosphere 60, 1034–1044.

Duncan, A.(1988) The ecology of slow sand filters.In proceedings: Slow sand filtration: recent development in water treatment technology.Chichester, UK.

Eighmy, T.T., Collins, M.R., Spanos, S.K., Fenstermacher, J (1992) Microbial populations, activities and carbon metabolism in slow sand filters. Water Research 26(10), 1319-1328.

Eighmy, T.T., Collins, M.R., Malley, J.P., Royce, J., Morgan, D. (1993) Biologically Enhanced Slow Sand Filtration for Removal of Natural Organic Matter. AWWARF, Denver, CO.

Eighmy, T.T., Spanos, S.K., Royce, J., Collins, M.R., Malley, J.P. (1994) Microbial activity in slow sand filters.In proceedings: Slow sand filtration. AWWA, Denver, CO

Esiobu, N., Armenta, L., Ike, J. (2002) Antibiotic resistance in soil and water environments. International Journal of Environmental Health Research 12(2), 133-144.

Flaherty, C.M. Dodson, S.I. (2005) Effects of pharmaceuticals on *Daphnia* survival, growth, and reproduction. Chemosphere 61(2), 200-207.

Flemming, H.-C., Schmitt, J., Marshall, K. C. (1996) In *Sorption properties of biofilms*. *Sediments Toxicology Substances*; Calmano, W., Foerstner, U., Eds.; Springer: Berlin, Germany, pp 115-157.

Flemming, C.A., Palmer, R.J., Arrage, A.A., Van der Mei, H.C., White, D.C. (1998) Cell surface physicochemistry alters biofilm development of Pseudomonas aeruginosa lipopolysaccharide mutants. Biofouling 13(3), 213-231.

Fonseca, A.C.; Summers, R.S.; Hernandez, M.T. (2001) Comparative measurements of microbial activity in drinking water biofilters. Water Research 36(16), 3817-3824.

Gagnon, G.A., Huck, P.M. (2001) Removal of easily biodegradable organic compounds by drinking water biofilms: analysis of kinetics and mass transfer. Water Research 35(10):2554-2564.

Gavalchin, J., Katz, S.E. (1994) The persistence of fecal-borne antibiotics in soil. Journal of the Association of Official Analytical Communities International 77, 481-485.

Ghosh, G.C., Okuda, T., Yamashita, N. (2009) Occurrence and elimination of antibiotics at four sewage treatment plants in Japan and their effects on bacterial ammonia oxidation. Water Science and Technology 59(4), 779-786.

Giger, W., Alder, A.C., Golet, E.M., Kohler, H.P.E., McArdell, C.S., Molnar, E., Siegrist, H., Suter, M.J.F. (2003) Occurrence and fate of antibiotics as trace contaminants in wastewaters, sewage sludges, and surface waters. Chimia 57(9), 485-491.

Gjaltema, A., Arts, P.A.M., van Loosdrecht, M.C.M., Kuenen, J.G., Heijnen, J.J. (1994). Heterogeneity of biofilms in rotating annular reactors: occurrence, structure, and consequences. Biotechnology and Bioengineering 44:2:194-204.

Göbel, A., McArdell, C.S., Suter, M.J-F., Giger, W. (2004) Trace determination of macrolide and sulfonamide antimicrobials, a human sulfonamide metabolite, and trimethoprim in wastewater using liquid chromatography coupled to electrospray tandem mass spectrometry. Analytical Chemistry 76(16) 4756.

Göbel, A., Thomsen, A., McArdell, C.S., Joss, A., Giger, W. (2005) Occurrence and sorption behaviour of sulfnonamides, macrolides and trimethoprim in activated sludge treatment. Environmental Science & Technology 39 (11), 3981–3989.

Grigorescu, A.S. Hozalski, R.M. (2010) Modeling HAA biodegradation in biofilters and water distribution systems. Journal AWWA 102(7), 67-80.

Grischek, T. (1999) Field Studies into the Attenuation of Contaminants During Riverbank Filtration. In. proc. International Riverbank Filtration Processes. Louisville, KY.

Grünheid, S., Amy, G., Jekel, M.(2005) Removal of bulk dissolved organic carbon (DOC) and trace organic compounds by bank filtration and artificial recharge. Water Research 39(14) 3219–3228.

Guibaud, G., Comte, S., Bordas, F., Dupuy, S., Baudu, M. (2005) Comparison of the complexation potential of extracellular polymeric substances (EPS), extracted from activated sludges and produced by pure bacteria strains, for cadmium, lead and nickel. Chemosphere 59(5), 629-638.

Haberer, K.; Drews, M; Waltenberg, M. (1984) Entfernung organischer Wasserinhaltstoffe bei der Langsamsandfiltration. Vom Wasser 63, 121-140.

Halling-Sorensen, B. (2000) Algal toxicity of antimicrobial agents used intensive farming. Chemosphere 40(7), 731-739.

Halling-Sorensen, B., Holten Lutzhoft, H.-C., Anderson, H.R., Ingerslev, F. (2000) Environmental risk assessment of antibiotics: comparison of mecillinam, trimethoprim and ciprofloxacin. Journal of Antimicrobial Chemotherapy 46(suppl. 1): 53-58.

Hansch, C., Leo, A. Hoekman, D. (1995) Exploring QSAR: Volume 2: Hydrophobic, Electronic, and Steric Constants. American Chemical Society, Washington, D.C.

Headley, J.V., Gandrass, J., Kuballa, J., Peru, K.M., Gong, Y. (1998) Rates of Sorption and Partitioning of Contaminants in River Biofilm. Environmental Science & Technology 32(24), 3968-3973.

Heberer, T., Mechlinski, A., Fanck, B., Knappe, A., Massmann, G., Pekdeger, A. Fritz, B. (2004) Field studies on the fate and transport of pharmaceutical residues in bank filtration. Ground water Monitoring & Remediation 24(2), 70-77.

Heberer, T., Massmann, G., Fanck, B., Taute, T. Dünnbier, U. (2008) Behaviour and redox sensitivity of antimicrobial residues during bank filtration. Chemosphere 73(4), 451-460.

Hirsch, R., Ternes, T., Haberer, K., Kratz, K.L. (1999) Occurrence of antibiotics in the aquatic environment. Science of the Total Environment 225(1-2):109-118.

Hiscock, K.M., Grischek, T (2002) Attenuation of groundwater pollution by bank filtration. Journal of Hydrology, 266(3-4), 139-144.

Hokanson, D.R., Zhang, Q., Cowden, JR., Troschinetz, A.M., Mihelcic, J.R., Johnson, D.M. (2007) Challenges to implementing drinking water technologies in developing world countries. Environmental Engineer: Applied Research & Practice 1, 2-9.

Hou, J.P., Poole, J.W. (1969) Kinetics and mechanisms of degradation of ampicillan in solution. Journal of Pharmaceutical Sciences 58(4): 447-454.

Hoyle, B.D. Costerton, J.W. (1991) Bacterial resistance to antibiotics: the role of biofilms. Progress in Drug Research 37, 91–105.

Huang, C.H., Renew, J.E., Smeby, K.L., Pinkston, K., Sedlak, D.L. (2001) Assessment of potential antibiotic contaminants in water and preliminary occurrence analysis, proceedings of the 2nd International Conference on Pharmaceuticals and Endocrine Disrupting Chemicals in Water, National Ground Water Association, Minneapolis, MN.

Huber, M. M., Korhonen, S., Ternes, T. A., von Gunten, U. (2005) Oxidation of pharmaceuticals during water treatment with chlorine dioxide. Water Research 39(15), 3607-3617.

Ingerslev, F., Halling-Sørensen, B. (2000) Biodegradability properties of sulfonamides in activated sludge. Environmental Toxicology and Chemistry 19(10), 2467-2473.

Jahn, A. Nielsen P.H. (1995) Extraction of extracellular polymeric substances (EPS) from biofilms using a cation exchange resin. Water Science & Technology 32(8), 157-164.

Jekel, M., Grischek, T. (2003) Riverbank Filtration: The European Experience. In proc. 2nd Annual Riverbank Filtration Conference, Cincinnati, OH, USA

Kawamura S. (1991) *Integrated Design of Water Treatment Facilities*. John Wiley and Sons, New York, NY, USA.

Kennedy A.F. Sutherland I.W. (1987) Analysis of bacterial exopolysaccharides. Biotechnology and Applied Biochemistry 9(1), 12–19.

Kim, S-.C., Carlson, K. (2007) Temporal and spatial trends in the occurrence of human and veterinary antibiotics in aqueous and river sediment matrices. Environmental Science & Technology 41(1), 50-57.

Kim, S.D.; Cho, J.;Kim, I.S.; Vanderford, B.J.; Snyder, S.A. (2007) Occurrence and removal of pharmaceuticals and endocrine disruptors in South Korean surface, drinking, and waste water Water Research 41(5), 1013-1021.

Knapp, C.W., Engemann, C.A., Hanson, M.L., Keen, P.L., Hall, K.J. and Graham, D.W. (2008) Indirect evidence of transposon-mediated selection of antibiotic resistance genes in aquatic systems at low-level oxytetracycline exposures. Environmental. Science and Technology 42, 5348–5353.

Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., Buxton, H.T. (2002) Pharmaceuticals, hormones, and other organic wastewater constituents in US streams, 1999-2000: a national reconnaissance. Environmental Science and Technology 36(6), 1202-1211.

Kuehn W, Mueller U. (2000) Riverbank filtration: an overview. Journal AWWA 92(12), 60-69.

Kuo, C-Y. (1998) Improved application of ion chromatographic determination of carboxylic acids in ozonated drinking water. Journal of Chromatography A 804, 265–272.

Lee, S.M., Davis, A.P. (2001) Removal of Cu (II) and Cd (II) from aqueous solution by seafood processing waste. Water Research 35(2), 534-540.

Li, B., Zhang, T. (2010) Biodegradation and Adsorption of Antibiotics in the Activated Sludge Process. Environmental Science & Technology 44(9), 3468-3473.

Liao, B.Q., Allen, D.G., Droppo, I.G., Leppard, G.G., Liss, S.N. (2001) Surface Properties of sludge and their role in bioflocculation and settleability. Water Research 35(2), 339-350.

Lin, A.Y.-C., Yu, T.-H., Lateef, S.K. (2009) Removal of pharmaceuticals in secondary wastewater treatment processes in Taiwan. Journal of Hazardous Materials 167(1-3), 1163-1169.

Lindberg, R., Jarnheimer, P-A., Olsen, B., Johansson, M.I., Tysklind, M. (2004) Determination of antibiotic substances in hospital sewage water using solid phase extraction and liquid chromatography/mass spectrometry and group analogue internal standards. Chemosphere 57(10), 1479-1488.

Liu, H., Fang, H.P. (2002) Characterization of electrostatic binding sites of extracellular polymers by linear programming analysis of titration data. Biotechnology and Bioengineering 80(7), 806-811.

Logsdon, G.S., ed. (1991) *Slow Sand Filtration* ASCE Environmental Engineering Water Supply Committee. ASCE, Reston, VA, USA.

Loke, M.L., Tjornelund, J., Halling-Sorensen, B. (2002) Determination of the distribution coefficient (log K_d) of oxytetracycline, tylosin A, olaquindox and metronidazole in manure. Chemosphere, 2002. 48(3), 351-361.

Lorian, V., Ed. (1996) Antibiotics in Laboratory Medicine, 4th ed. Lippincott Williams & Wilkins, Baltimore, MD.

Martin, R.E. (1990) Quantitative description of bacterial deposition and initial biofilm development in porous media. Ph.D. Dissertation. The Johns Hopkins University, Baltimore, MD.

Massmann, G., Dünnbier, U., Heberer, T., Taute, T. (2008) Behaviour and redox sensitivity of pharmaceutical residues during bank filtration – Investigation of residues of phenazone-type analgesis. Chemosphere 71(8), 1476-1485.

McArdell, C.S., Molnar, E., Suter, M.J., Giger, W. (2003) Occurrence and fate of macrolide antibiotics in wastewater treatment plants and in the Glatt Valley watershed, Switzerland. Environmental Science and Technology 37(24), 5479-5486.

McFarland, J.W., Berger, C.M., Froshauer, S.A., Hayashi, S.F., Hecker, S.J., Jaynes, B.H., Jefson, M.R., Kamicker, B.J., Lipinski, C.A., Lundy, K.M., Reese, C.P., Vu, C.P. (1997) Quantitative Structure−Activity Relationships among Macrolide Antibacterial Agents: In Vitro and in Vivo Potency against *Pasteurella multocida.* Journal of Medicinal Chemistry 40(9), 1340-1346.

Mellon, M., Benbrook, C. Benbrook, K.L. (2001) Hogging it: estimation of antimicrobial use in Livestock. Union of Concerned Scientists: Cambridge, MA.

Miao, S.X., Bishay, F., Chen, M., Metcalfe, C.D. (2004) Occurrence of antimicrobial in the final effluents of wastewater treatment plants in Canada. Environmental Science and Technology 38(13), 3533-3541.

Monzón, M.; Oteiza, C.; Leiva, J, Amorena, B. (2001) Synergy of different antibiotic combinations in biofilms of *Staphylococcus epidermidis*. Journal of Antimicrobial Chemotherapy 48, 793-801.

Moore, D.E., Zhou, W. (1994) Photodegradation of sulfamethoxazole. A chemical system capable of monitoring seasonal changes in UVB intensity. Photochemistry and Photobiology 59(5), 497-502.

Muñoz-Aguayo, J., Lang, K.S., LaPara, T.M., González, G. Singer, R.S. (2007) Evaluating the effects of chlortetracycline on the proliferation of antibiotic-resistant bacteria in a simulated river water ecosystem. Applied Environmental Microbiology 73(17), 5421-5425.

Naeem, S., Li, S. (1997) Biodiversity enhances ecosystem reliability. Nature 390, 507-509.

Nakada, H., Shinohara, H., Murata, A., Kiri, K., Managaki, S., Sato, N., Takada, H. (2007) Removal of selected pharmaceuticals and personal care products (PPCPs) and endocrine-disrupting chemicals (EDCs) during sand filtration and ozonation at a municipal sewage treatment plant Water Research 41(19), 373-4382.

Naslund, J., Hedman, J.E. Agestrand, C. (2008) Effects of the antibiotic ciprofloxacin on the bacterial community structure and degradation of pyrene in marine sediment. Aquatic Toxicology 90, 223–227.

Nelson, D.K., LaPara, T.M. Novak, P.J. (2010) Effects of ethanol-based fuel contamination: microbial community changes, production of regulated compounds, and methane generation. Environmental Science and Technology 44(12), 4525-4530.

Neu, H.C. (1991) Synergy and antagonism of combinations with quinolones. European Journal of Microbiology and Infectious Diseases 10(4), 255-261.

Ngheim, L.D., Schäfer, A.I., Elimelech, M. (2005) Pharmaceutical retention mechanisms by nanofiltration membranes. Environmental Science & Technology 39(19), 7698-7705.

Nowara, A., Burhenne, J., Spiteller, M. (1997) Binding of fluoroquinolone carboxylic acid derivatives to clay minerals. Journal of Agricultural Food Chemistry 45(4):1459-1463.

Onan, L.J., T.M. LaPara. (2003) Tylosin-resistant bacteria cultivated from agricultural soil. FEMS Microbiology Letters 220(1):15-20.

O'Shea R., Moser, H.E. (2008) Physicochemical properties of antibacterial compounds: implications for drug discovery. Journal of Medicinal Chemistry 51(10), 2871-2878.

Owen, D.M, Amy, G.L., Chowdhury, Z.K., Paode, R., McCoy, G., Viscosil, K. (1995) NOM characterization and treatability. Journal AWWA 87(1), 46-63.

Pan, X.S., Ambler, J., Mehtar, S., Fisher, L.M. (1996) Involvement of topoisomerase IV and DNA gyrase as ciprofloxacin targets in Streptococcus pneumonia. Antimicrobial Agents and Chemotherapy. 40(10), 2321-2316.

Pascaud, A., Amellal, S., Soulas M.L., Soulas, G. (2009) A fluorescence-based assay for measuring the viable cell concentration of mixed microbial communities in soil. Journal of Microbiological Methods 76(1), 81-87.

Pena, A., Chmielova, D., Lino, C.M. Solich, P. (2007) Determination of fluorquinolone antibiotics in surface waters from Mondego River by high performance liquid chromatography using amonolithic column. Journal of Separation Science 30, 2924-2928.

Prankerd, R.J. (2007) *Profiles of Drug Substances, Excipients and Related Methodology, Volume 33: Critical Compilation of pKa Values for Pharmaceutical Substances (Analytical, Excipients, and Related Methodology)* Academic Press, Amsterdam, Netherlands

Qiang, Z., Adams, C. (2004) Potentiometric determination of acid dissociation constants (pK_a) for human and veterinary antibiotics. Water Research 38(12), 2874-2890.

Rabolle, M., Spliid, N.H. (2000) Sorption and mobility of metrondazole, olaquindox, oxytetracycline and tylosin in soil. Chemosphere 40(7), 715-722.

Rachid, S., Ohlsen, K., Witte, W. Hacker, J. (2000) Effect of subinhibitory antibiotic concentrations on polysaccharide intercellular adhesin expression in biofilm-forming *Staphylococcus epidermidis*. Antimicrobial Agents and Chemotherapy 44(12), 3357–3363.

Rachwal, A.J., Bauer, M.J., Chipps, J.S., Colbourne, J.S., Foster, D.M. (1996) Comparisons between slow sand and high rate biofiltration. In proceedings: Third International Slow Sand/Alternative Biofiltration Conference. John Wiley and Sons, NY, USA.

Radjenovića, J., Petrović, M., Barcelóa, D., Petrović, M. (2007) Advanced mass spectrometric methods applied to the study of fate and removal of pharmaceuticals in wastewater treatment. Trends in Analytical Chemistry 26(11), 1132-1144.

Radke, M., Lauwigi, C., Heinkele, G., Murdter, T.E., Letzel, M. (2009) Fate of the antibiotic sulfamethoxazole and its two major human metabolites in a mater sediment test. Environmental Science and Technology 43 (9), 3135-3141.

Renew, J.E., Huang, C.H. (2004) Simultaneous determination of fluoroquinolone, sulfonamide, and trimethoprim antibiotics in wastewater using tandem solid phase extraction and liquid chromatography-electrospray mass spectrometry. Journal of Chromatography A 1042(1-2), 113-121 .

Rittmann, B.E., Crawford, L., Tuck, C.K. Narnkung, E. (1986) In situ determination of kinetic parameters for biofilms: isolation and characterization of oligotrophic biofilms Biotechnology and Bioengineering 28, 1753-1760.

Rooklidge, S.J. (2004) Antimicrobial Contaminant Removal by Mulit-stage Drinking Water Filtration. PhD Dissertation, Oregon State University, Corvallis, OR.

Rooklidge, S.J., Burns, E.R., Bolte, J.P. (2005) Modeling Antimicrobial contaminant removal in slow sand filtration. Water Research 39(2-3), 331-339.

Rooklidge, S.J., Miner, JR., Kassim, T.A. Nelson, P.O. (2005) Antimicrobial contaminant removal by multistage slow sand filtration. Journal AWWA 97(12), 92-100.

Schwarzenbach, R.P., Gschwend, P.M., Imboden, D. (2003) *Environmental Organic Chemistry, 2nd Edition*, Wiley Interscience, New Jersey.

Schwartz, T., Kohnen, W., Jansen, B., Obst, U. (2003) Detection of antibiotic-resistant bacteria and their resistance genes in wastewater, surface water, and drinking water biofilms. FEMS Microbiology Ecology 43(3), 325-335.

Scribner, E.A.; Battaglin, W.A.; Dietze, J.E.; Thurman, E.M.. Reconnaissance data for glyphosphate, other selected herbicides, their degradation products, and antibiotics in 51 streams in nine midwestern states, 2002. U.S. Geological Survey: Lawrence, Kansas

Snyder, S.A., Adham, S., Redding, A.M., Cannon, F.S., DeCarolis, J., Oppenheimer, J., Yoon, Y. (2007) Role of membranes and activated carbon in the removal of endocrine disruptors and pharmaceuticals. Desalination 202(1-3), 156-181.

Suarez, S., Lema, J.M., Omil, F. (2010) Removal of pharmaceutical and personal care products (PPCPs) under nitrifying and denitrifying conditions. Water Research 44(10), 3214-3224.

Takács-Novák, K., Noszál, B., Tokes-Kovesdi, M., Szasz, G., (1993) Acid base properties and proton-speciation of vancomycin. International Journal of Pharmacy89(3), 261-263.

Tenson, T., Lovmar, M., Ehrenberg, M. (2003) The mechanism of action of macrolides, linosamides, and streptogramin B reveals the mascent peptide exit path in the ribosome. Journal of Molecular Biology. 330(5), 1005-1014.

Thiele-Bruhn, S. (2003) Pharmaceutical antibiotic compounds in soil – a review. Journal of Plant Nutrition and Soil Science 166(2), 145-167.

Thiele-Bruhn, S., Seibicke, T., Schulten, H.R., Leinweber, P. (2004) Sorption of sulfonamide pharmaceutical antibiotics on whole soils and particle-size fractions. Journal of Environmental Quality 33(4), 1331-1342.

Thiele-Bruhn, S. (2005) Microbial inhibition by pharmaceutical antibiotics in different soils—dose-response relations determined with the iron (III) reduction test. Environmental Toxicology and Chemistry 24(4), 869-876.

Thiele-Bruhn, S., Beck, C. (2005) Effects of sulfonamide and tetracycline antibiotics on soil microbial activity and microbial biomass. Chemosphere 59, 457-465.

Tolls, J., Gebbink, W., Cavallo, R. (2002) pH-dependence of sulfonamide antibiotic sorption: data and model and evaluation. In proceedings: SETAC Europe 12th Annual Meeting, Vienna.

Torniainen, K., Mattinen, J., Askolin, C.P., Tammilehto, S. (1997) Structure elucidation of a photodegradation product of ciprofloxacin. Journal of Pharmaceutical and Biomedical Analysis 15(7), 887-894.

USEPA. (1996) OPPTS 830.7550 Partition Coefficient (n-octanol/water), shake flask method. EPA 712-C-96-038, Washington, D.C.

Volmer, D.A., Hui, J.P.M. (1998) Study of erythromycin A decomposition products in aqueous solution by solid-phase microextraction/liquid chromatography/tandem mass spectrometry. Rapid Communications in Mass Spectrophotometry 12(3),123-129.

von Canstein, H.V., Kelly, S., Li, Y., Wagner-Dobler, I. (2002) Species diversity improves the efficiency of mercury-reducing biofilms under changing environmental conditions. Applied Environmental Microbiology 68(6), 2829-2837.

Wang, J, Hubbs, S., Song, R. (2002) Evaluation of Riverbank Filtration as a Drinking Water Treatment Process. AWWARF. Denver, CO.

Weber-Shirk, M., Dick, R.I. (1997) Physical-chemical mechanisms in slow sand filters. Journal AWWA 89(1), 87-100.

Weiss, W.J., Bouwer, E.J., Ball, W.P., O'Melia, C.R. Arora, H., Speth, T.F. (2003) Comparing RBF with bench-scale conventional treatment for precursor reduction. Journal of the American Waterworks Association 95(12), 67-80.

Westerhoff, P., Yoon, Y., Snyder, S., Wert, E. (2005) Fate of endocrine-disruptor, pharmaceutical, and personal care product chemicals during simulated drinking water treatment processes. Environmental Science & Technology, 39(17), 6649-6663.

Wicke, D., Böckelmann, U., Reemtsma, T. (2007) Experimental and modeling approach to study sorption of dissolved hydrophobic organic contaminants to microbial biofilms. Water Research 41(10), 2201-2210.

Wiegel, S., Aulinger, A., Brockmeyer, R., Harms, H., Löffler, J., Reincke, H. Schmidt, R.Stachel, B., von Tümpling, W., Wanke, A. (2004) Pharmaceuticals in the river Elbe and its tributaries. Chemosphere 57(2), 107-126.

Worch, E., Grischek, T., Boernick, H., Eppinger, P. (2002) Laboratory tests for simulating attenuation processes of aromatic amines in riverbank filtration. Journal of Hydrology 266(3-4): 259-268.

Wu, C., Spongberg, A.L., Witter, J.D. (2009) Sorption and biodegradation of selected antibiotics in biosolids. Journal of Environmental Science and Health Part A 44(5), 454-461.

Yang, Li-H., Ying, G-G., Su, H.C., Stauber, J.L., Adams, M.S., Binet, M.T. (2008) Growth-inhibiting effects of 12 antibacterial agents and their mixtures on the freshwater microalga *Pseudokirchneriella subcapitata*. Environmental Toxicology and Chemistry 27(5), 1201–1208.

Xu, W.H., Zhang, G., Wai, O.W.H., Zou, S.C., Li, X.D. (2009) Transport and adsorption of antibiotics by marine sediments in a dynamic environment. Journal of Soils and Sediments 9, 364–373.

Yang, Li-.H., Ying, G-.G., Su, H-,C., Stauber, J.L., Adams, M.S., Binet, M.T. (2008) Growth-inhibiting effects of 12 antibacterial agents and their mixtures on the freshwater microalga *Pseudokirchneriella subcapitata*. Environmental Toxicology and Chemistry 27(5), 1201–1208.

Yeager, R.L., Halley, B.A. (1990) Sorption/Desorption of [^{14}C]efrotymycin with soils. Journal of Agricultural Food Chemistry 38(3), 883-886.

Ying, G-G, Kookana, R.S., Dillion, P. (2003) Sorption and degradation of selected five endocrine disrupting chemicals in aquifer material. Water Research 37(15)3785-3791.

Yordanov, R.V., Lamb, A.J., Melvin, M.A.L., Littlehohn, J. (1996) Biomass characteristics of slow sand filters receiving ozonated water. In proceedings: Advances in slow sand and alternative biological filtration. UK: Wiley.

Zabinski, R.A.; Walker, K.J.; Larsson, A.J.; Moody, J.A.; Kaatz, G.W.; Rotshafer, J.C. Effect of aerobic and anaerobic environments on antistaphylococcal activities of five fluoroquinolones. Antimicrobial Agents and Chemotherapy, 1995, 39:2:507-512.

Zhang, P., LaPara, T.M., Goslan, E., Xie, Y., Parsons, S. Hozalski, R.M. (2009) Biodegradation of haloacetic acids by bacterial isolates and enrichment cultures from drinking water systems. Environmental Science and Technology, 43(9), 3169–3175.

Appendix A. ARISA data for objective 1 inhibition experiments

Table A.1. Fragment Length versus % Total Area for CFRAB biofilm samples

sample\fragment length (bp)	475	555	578	627	646	657	670	695	718	725	732	744	757	769	793
Control (1)	2.5	1.7	2.3			1.0								92.6	
Control (2)	6.2			2.6			1.6	1.8				4.7	22.1	61.0	
3.33 all (1)				13.1			28.6			6.2			42.5	9.7	
3.33 all (2)				37.8								2.7	53.6	5.9	
3.33 CIP, 0.33 SMX & ERY (1)	2.6		3.5	20.1	4.7		6.3	13.3	9.5			27.1	5.4		7.6
3.33 CIP, 0.33 SMX & ERY (2)	3.4		6.5	12.8	7.0		7.0				40.5	14.8	1.0		7.0
0.33 all (1)	1.4		1.6					14.7			42.7			39.6	
0.33 all (2)	3.8		5.9	13.9				4.8					11.9	59.6	
3.33 SMX, 0.33 CIP & ERY	5.3		17.8											76.9	
3.33 ERY, 0.33 SMX & CIP	5.4		5.7	11.3										77.6	

Appendix B. Further detail on acetate sampling and analysis

Sample Collection

CFRAB effluent samples for acetate analysis were collected in prewashed 130 mL amber glass bottles, with benzalkonium chloride pre-added to the bottles for a final concentration of 30 mg/L as a preservative. The water samples are then filtered using syringe filters (Millipore 0.45 μm nylon syringe filters) and 60 mL syringes (BD Syringe, Luer-Lok Tip) into 60 mL Nalgene bottles and refrigerated at 4°C until analysis.

Analysis

Ion chromatography (IC) is used for the analysis of acetate. The method for acetate analysis was adapted from that of Kuo (1998). A Dionex OnGuard-H^+ hydrogen cartridge was used for sample preparation during transfer from the 60 mL bottles to the 5 mL polyvials used for sample loading to the autosampler, All sample bottles and IC polyvials were pre-washed (2x) in ultra-pure water according to the instructions provided by Dionex. The IC system (Dionex DX500) includes an autosampler (AS40), an electrochemical detector (ED40), an oven (LC25), a gradient pump (GP40) and a suppressor (ASRS 300) operated in external water mode at 3 p.s.i. with the current controller set to 50 mA. A 50 μL sample loop is used. Samples are fed through a AG 11 (4 x 50 mm) guard column prior to the AS11 (4 x 250 mm) analytical column.Three eluents were prepared for gradient mode operation. Stock eluents include 1 mM NaOH, 100 mM NaOH, and degassed nanopure water. An ATC-3 (4 mm) anion trap is used for all eluent feeds. The working eluents were fed as follows:

- Isocratic mode for 5 minutes; approx. 0.2 mM NaOH;
- Gradient mode 30 minutes; linear increase from approx. 0.2 mM to 20 mM NaOH;
- Stabilizing mode 15 minutes; approx 0.2 mM NaOH

Acetate standards at 25 μg/L, 100 μg/L, 200 μg/L and 400 μg/L (OAc^-) are prepared for each run in the minimal medium solution and preserved with benzalkonium chloride at 30 mg/L. Correlation coefficient (R^2) values have been greater than 0.995 for standard calibration curves (with duplicate samples) for all IC runs.

Immediately before analysis, standards and samples were pretreated with the Dionex OnGuard-H^+ hydrogen cartridge. A cartridge was prepared by injecting 10 mL of nanopure water at 2 mL/min through it. Samples were then prepared with injecting 10 mL of sample through the hydrogen cartridge, wasting the first 5 mL. The cartridge was disposed of after preparing one set of standards/samples (~20). The same 5 mL syringe was used for all samples, triple-washing with nanopure water in between samples. Pretreating with the hydrogen cartridge greatly improves the sharpness of the acetate peak and chromatogram area.

Before running the samples through the IC, the IC is base-lined using a feed containing 20 mM NaOH until readings were stable in a 0.5 μS range. Base-lining the IC requires approximately 2 to 2½ hours. The following maintenance procedures were carried out after each run to ensure low baseline conductivity levels:

- The ATC was disconnected from the columns and 2.0 M NaOH was pumped through it at 1 mL/min for 100 min, followed by 0.2 mM NaOH for 20 min.
- 3 mL of 2.0 N sulfuric acid followed by 3 mL of nanopure water was injected into the ASRS through the "Eluent Out" port. Then 5mL of 2.0N sulfuric acid followed by 5 mL of nanopure water was injected into the ASRS through the "Regenerant In" port. Effluent from the ASRS was directed to a waste line.
- 10 mL of deionized water was injected into each of the pump heads.

Additionally, the guard and analytical columns become contaminated over time (3-4 weeks of frequent IC runs), resulting in a delay in the acetate peak (by about 30 seconds) and decreased peak resolution. When contamination is evident, the ATC and ASRS are disconnected from columns and the analytical column is placed before the guard column. A cleanup solution of 200 mM hydrochloric acid solution in 80 volume% acetonitrile (remainder nanopure water) is prepared and used immediately (since the solution degrades quickly). The columns are rinsed with nanopure water for 10 min, followed by the cleanup solution for 60 min, then nanopure water for another 10 min, and finally 0.2 mM for 60 min. All flowrates are at 1 mL/min.

REFERENCES

Kuo, C-Y. Improved application of ion chromatographic determination of carboxylic acids in ozonated drinking water. Journal of Chromatography A, 1998. 804:265

Notes of Preparation and Quantitation of Acetate

Preparation of Acetate Stock Solutions
Acetate stock solutions of 100 mg/L were prepared using sodium acetate and ultrapure water. Stock solutions were considered stable for three months. Purity of the sodium acetate was taken into account when calculating the mass needed for the solution.

Preparation of Acetate Standard Solutions
Acetate standard solutions were prepared in a 50 mL volumetric flask using an acetate stock solution of 100 mg/L, deionized water, and mineral water. Stock solution was measured using an autopipet and placed in the flask. 15.625 mL of deionized water (not Type 1) was added using an autopipet. Mineral water A (the same water used as water feed to columns) with benzalkonium chloride (52.5 mg/L, prepared with 3.5mL 15g/L benzalkonium chloride and mineral water) was then added to the stock solution until the 50 mL level in the volumetric flask was reached.

Acetate standard solutions of 50, 100, 150, 200, 250, 750, 1250, and 2000 mg/L were prepared. The quantity of stock solution required is shown in Table B.1 below. Note: Autopipets are most accurate above 50% of the rated capacity. For preparation of standards, do not use an autopipet for a volume below 50% of rated capacity.

Table B.1: Stock Solution Required for Preparation of Acetate Standard Solutions

Acetate Standard (µg/L)	Acetate in 50 mL solution (µg)	Volume Stock Solution (µL)
25	1.25	12.5
50	2.5	25.0
100	5.0	50.0
150	7.5	75.0
200	10.0	100.0
2 50	12.5	125.0
750	37.5	375.0
1250	62.5	625.0
2000	100.0	1000.0

Preparation of Eluent
Sodium hydroxide (NaOH) eluents of 1mM and 100mM were prepared from Type 1 water and 50wt% sodium hydroxide solution (NaOH bottle stored in a decanter). NaOH was added to a 2000mL volumetric flask using an autopipette. The same pipette tip was then filled with Type 1 water and the contents pipetted into the volumetric flask to flush out all the NaOH. 104uL of 50wt% NaOH was used to produce the 1mM solution while 10.4mL was used for the 100mM solution. Type 1 water was then added to the mark. The bottle of NaOH was flushed with nitrogen gas and replaced in the decanter, which was also flushed with nitrogen gas. The resulting eluent was then degassed.
Preparation of Anion Trap Regenerant

52mL of 50wt% sodium hydroxide was measured in a graduated cylinder and poured into a 500mL beaker. Deionized water was added until to the 500mL mark.

Preparation of Suppressor Cleaner

0.562 mL of 95-98% sulfuric acid was added to a 100 mL volumetric flask using a micropipette. The same pipette tip was then filled with Type 1 water and the contents pipetted into the volumetric flask to flush out all the sulfuric acid. Type 1 water was then added to the mark.

Preparation of Column Regenerant

3.33 mL of hydrochloric acid was added to a 100 mL volumetric flask using a micropipette. The same pipette tip was then used to pipet 16.66 mL of Type 1 water into the same volumetric flask. Acetonitrile was then added to the mark.

Operating the Ion Chromatograph

I. Standards, Eluent, ATC Regenerant , Water, and Sulfuric Acid Preparation

New standards (acetate in mineral water) should be prepared as needed. Always have fresh stocks of eluent (NaOH 1 mM and 100 mM) on hand. New ATC regenerant (NaOH 2.0 M) should be prepared if less than 100 mL remains. See solution preparation folder for details. Approximately 4 liters of degassed Type 1 water will need to be prepared for every run. Prepare sulfuric acid whenever it runs out. See solution preparation folder for details.

II. Creating Schedules

The first sample should always be a blank (Type 1 water). Use “Sample” for type and “Blank” for method. Each other sample should use “Wunder Anion” for method. Schedules can be made by creating a copy of an old schedule. Look under C: -> Peaknet -> Wunder. Create a new folder to store results for each run (runs have been stored under C: -> Peaknet -> Wunder -> *date*), and make sure to assign the right save-location to each sample when creating the schedule.

III. Washing and Filling Polyvials

Polyvials need to be washed twice with Type 1 water to prevent leeching. Fill the polyvials with Type 1 water and soak for 4 hours then empty and fill again for 24 hours. Empty and leave to dry, and then seal in a zip lock bag until needed.

Samples need to be treated with a hydrogen cartridge before being run through the IC. Take a fresh hydrogen cartridge and prepare it by injecting 10mL of Type 1 water though it at less than 2 mL per minute using a fresh syringe. Note that very little pressure is required to drive the water through the cartridge.

Using the same syringe, draw 5 mL of sample and inject it into the hydrogen cartridge under 2 mL per minute and discard the sample. Using the same syringe, draw another 5 mL of the same sample and inject it at the same rate, directing the sample into a polyvial. Triple-wash the syringe with Type 1 water, and repeat for each sample.

The first polyvial to be run through the IC should be filled with 5 mL of Type 1 water (no need to inject into the hydrogen cartridge).

IV. Base-lining and Running the IC

Load the schedule and press "baseline". Ensure that degassed Type 1 water runs through the suppressor at 1-2 mL per minute (pressure ~ 25 psi, but varies – check with a stopwatch and graduated cylinder). On the computer, there should be a graph of conductivity vs. time. Adjust the scale on the graph so that it is 0.3 μS on the y-axis. Allow the IC to baseline until conductivity readings stay within that range (about 1½-3 hours). Expect substantial noise in the data.

V. Maintenance

a. ATC

Place the feed tubing for Eluent A in 2.0M NaOH and check that the eluent bottles are not empty. Disconnect the ATC-3 from the injection valve and attach that end to a waste line. Switch the pump from remote to local operation and from direct control to method control. Select method 14 and run for two hours. Reconnect the ATC-3 to the injection valve.

Conduct ATC maintenance at the end of every run.

b. Suppressor

Disconnect the "Eluent in" tubing and "Eluent out tubing." Connect a waste line to the "Eluent in" port. Set up a temporary line to the "Eluent out" port and inject 3 mL of 0.2N sulfuric acid followed by 3 mL of Type 1 water. (Expect a lot of resistance, be careful of syringe disconnecting from tubing). Reconnect original tubing.

Disconnect the "Regenerant in" tubing and set up a temporary line. Ensure that there is a waste line from the "Regenerant out" port. Inject 5 mL of 0.2N sulfuric acid followed by 5 mL of Type 1 water through the temporary line.

Conduct suppressor maintenance at the end of every run.

c. Pump Head

Using a syringe inject 10 mL of deionized or Type 1 water into the pump head to wash off salts accumulated over the run.

Conduct pump head maintenance at the end of every run.

VI. Trouble-shooting

a. Low-pressure pump warning while base-lining

   Check connection between ATC and injection valve

b. Drop in peak area for duplicated sample

   Possible guard (and analytical) column saturation. Clean guard column as per instructions in manual. If problem persists, clean analytical column also. If problem still persists, may be due to bacteria in samples - check that sufficient preservative is being used.

c. Backward-shift in eluting time

   Guard Column is contaminated. Clean guard column as per instructions in manual. If problem persists, clean analytical column also.

Appendix C. Acetate and VS data from objective 1 experiments

Table C.1 Acetate and VS data for Control (1)

6-Aug
Control (no antibiotics)

	Parameters	areal	
μ_{max} =	1.71	0.15	see units below
K_s =	0.0036	mg C/L	
R^2 =	0.978		
VS =	260	mg	
area =	2960	cm^3	

S (mg C/L)	acetate substrate utilization rate (mg C/mg VS/day)	areal acetate substrate utilization rate (mg C/cm^2/day)
0.008	1.107	0.097
0.008	1.107	0.097
0.009	1.107	0.097
0.013	1.476	0.130
0.015	1.476	0.130
0.015	1.475	0.130
0.617	1.734	0.152
0.712	1.716	0.151
0.789	1.702	0.150
2.929	1.676	0.147
2.944	1.673	0.147
3.007	1.662	0.146

slide	dried weight (g)	volatilized weight (g)	delta (mg)	mg VS	
1	1.23845	1.2361	2.35	248	
2	1.23849	1.23582	2.67	282	
3	1.23283	1.22992	2.91	308	
4	1.23773	1.23808			
5	1.25097	1.24869	2.28	241	
6	1.252	1.24957	2.43	257	
7	1.24246	1.24037	2.09	221	
8	1.25347	1.25112	2.35	248	
9	1.24878	1.24625	2.53	267	
10	1.2495	1.24775	1.75	185	
11	1.24394	1.24201	1.93	204	
12	1.22762	1.22552	2.1	222	
13	1.24792	1.24609	1.83	193	
14	1.25207	1.25009	1.98	209	
15	1.24424	1.24223	2.01	212	
control 1	1.24624	1.24669	-0.45	35	std dev
control 2	1.25775	1.25803	-0.28	9	std error
control 3	1.24743	1.24733	0.10		
control 4	1.24468	1.24497	-0.29		
control 5	1.25065	1.25087	-0.22		
		control adjustment	-0.228	mg VS	
		average biomass per slide	2.23	mg VS	
		biomass per slide (ajd.)	2.46	mg VS	
		surface area of each slide	28	cm^3	
		wetted surface area of reactor	2960	cm^3	
		reactor biomass	259.77	mg VS	

Table C.2 Acetate and VS data for Control (2)

18-Sep
Control (no antibiotics)

	Parameters	Areal	
μmax =	1.62	0.12	see units below
Ks =	0.0004	mg C/L	
R2 =	0.941		
VS =	219	mg	
area =	2960	cm^3	

S (mg C/L)	acetate substrate utilization rate (mg C/mg VS/day)	acetate substrate utilization rate (mg C/cm2/day)
0.001	1.313	0.097
0.006	1.312	0.097
0.008	1.312	0.097
0.122	1.725	0.128
0.133	1.722	0.127
0.159	1.717	0.127
2.202	1.707	0.126
2.476	1.647	0.122
2.889	1.557	0.115
4.620	1.616	0.120
4.778	1.581	0.117
4.868	1.562	0.116

slide	dried weight (g)	volatilized weight (g)	delta (mg)	mg VS	
1	1.23474	1.23263	2.11	223	
2	1.23478	1.23379	0.99	105	
3	1.23218	1.23052	1.66	175	
4	1.2402	1.2391	1.1	116	
5	1.23019	1.22862	1.57	166	
6	1.24412	1.24233	1.79	189	
7	1.24178	1.24024	1.54	163	
8	1.23534	1.23347	1.87	198	
9	1.24082	1.23828	2.54	269	
10	1.24037	1.23852	1.85	196	
11	1.24053	1.2386	1.93	204	
12	1.23932	1.23733	1.99	210	
13	1.2315	1.22956	1.94	205	
14	1.23622	1.23442	1.8	190	
15	1.22204	1.22045	1.59	168	
control 1	1.22253	1.2229	-0.37	40	std dev
control 2	1.22395	1.22391	0.04	10	std error
control 3	1.22158	1.22202	-0.44		
control 4	1.22547	1.22599	-0.52		
		control adjustment	-0.3225	mg VS	
		average biomass per slide	1.75	mg VS	
		biomass per slide (ajd.)	2.07	mg VS	
		surface area of each slide	28	cm^3	
		wetted surface area of reactor	2960	cm^3	
		reactor biomass	219.23	mg VS	

Table C.3 Acetate and VS data for 0.33 µg/L each antibitoic (1)

30-Jul

0.33 ug/L each antibiotic

	Parameters	areal		
µmax =	1.64		0.12	see units below
Ks =	0.0074	mg C/L		
R2 =	0.938			
VS =	225	mg		
area =	2960	cm^3		

S (mg C/L)	acetate substrate utilization rate (mg C/mg VS/day)	acetate substrate utilization rate (mg C/cm2/day)
0.011	0.985	0.075
0.011	0.984	0.075
0.013	0.985	0.075
0.024	1.311	0.100
0.024	1.310	0.100
3.572	1.470	0.112
3.639	1.469	0.112
3.696	1.489	0.113
15.510	1.732	0.132
15.920	1.758	0.134
16.423	1.896	0.144

Feed Condit 0.33 µg/L each antibiotic

slide	dried weight (g)	volatilized weight (g)	delta (mg)	mg VS	
1	1.24351	1.24192	1.59	168	
2	1.25097	1.2495	1.47	155	
3	1.2556	1.2535	2.1	222	
4	1.24995	1.2483	1.65	174	
5	1.24566	1.24426	1.4	148	
6	1.24995	1.24745	2.5	264	
7	1.2434	1.2416	1.8	190	
8	1.25401	1.25245	1.56	165	
9	1.2538	1.25184	1.96	207	
10	1.25473	1.25258	2.15	227	
11	1.25691	1.25488	2.03	215	
12	1.2445	1.24276	1.74	184	
13	1.25076	1.2476			
14	1.22461	1.22192	2.69	284	
15	1.24478	1.24246	2.32	245	
control 1	1.23833	1.23885	-0.52	42	std dev
control 2	1.25016	1.25035	-0.19	8	std error
control 3	1.24325	1.24338	-0.13		
control 4	1.24258	1.24279	-0.21		
control 5	1.24596	1.24593	0.03		
		control adjustment	-0.204	mg VS	
		average biomass per slide	1.93	mg VS	
		biomass per slide (ajd.)	2.13	mg VS	
		surface area of each slide	28	cm^3	
		wetted surface area of reactor	2960	cm^3	
		reactor biomass	225.14	mg VS	

Table C.4 Acetate and VS data for 0.33 µg/L each antibitoic (2)

13-Aug
0.33 ug/L each antibiotic

	Parameters	areal	
μ_{max} =	1.60	0.16	see units below
K_s =	0.0071	mg C/L	
R^2 =	0.959		
VS =	292	mg	
area =	2960	cm^3	

S (mg C/L)	acetate substrate utilization rate (mg C/mg VS/day)	acetate substrate utilization rate (mg C/cm²/day)
0.010	0.985	0.097
0.012	0.985	0.097
0.014	0.984	0.097
0.025	1.311	0.129
0.031	1.310	0.129
0.031	1.310	0.129
0.943	1.489	0.147
1.060	1.470	0.145
1.064	1.469	0.145
1.310	1.758	0.173
1.465	1.732	0.171

slide	dried weight (g)	volatilized weight (g)	delta (mg)	mg VS	
1	1.23667	1.2341	2.57	272	
2	1.2379	1.23467	3.23	341	
3	1.22945	1.2284	1.05	111	
4	1.23606	1.23272	3.34	353	
5	1.23537	1.23335	2.02	214	
6	1.24535	1.24294	2.41	255	
7	1.23617	1.23401	2.16	228	
8	1.23488	1.23293	1.95	206	
9	1.23278	1.23111	1.67	177	
10	1.23151	1.22981	1.7	180	
11	1.23329	1.23172	1.57	166	
12	1.22699	1.22385	3.14	332	
13	1.23548	1.23351	1.97	208	
14	1.21299	1.21186	1.13	119	
15	1.22936	1.22493	4.43	468	
control 1	1.22827	1.22874	-0.47	97	std dev
control 2	1.22711	1.22758	-0.47	25	std error
control 3	1.22772	1.22827	-0.55		
control 4	1.23164	1.23212	-0.48		
control 5	1.23314	1.23353	-0.39		
		control adjustm	-0.472	mg VSS	
		average biomas	2.29	mg VSS	
		biomass per sli	2.76	mg VSS	
		surface area of	28	cm^3	
		wetted surface	2960	cm^3	
		reactor biomass	291.91	mg VSS	

Table C.5 Acetate and VS data for 3.33 µg/L each antibitoic (1)

20-Aug
3.33 ug/L each antibiotic

	Parameters	areal	
μ_{max} =	1.27	0.13	see units below
K_s =	0.0094	mg C/L	
R^2 =	0.999		
VS =	308	mg	
area =	2960	cm^3	

S (mg C/L)	acetate substrate utilization rate (mg C/mg VS/day)	acetate substrate utilization rate (mg C/cm2/day)
0.025	0.931	0.097
0.026	0.931	0.097
0.254	1.207	0.126
0.265	1.205	0.125
0.282	1.203	0.125
1.813	1.276	0.133
1.851	1.270	0.132
1.876	1.266	0.132
3.755	1.285	0.134
3.882	1.265	0.132
3.956	1.253	0.130

slide	dried weight (g)	volatilized weight (g)	delta (mg)	mg VS	
1	1.23825	1.23537	2.88	304	
2	1.23745	1.23499	2.46	260	
3	1.2353	1.23335	1.95	206	
4	1.24509	1.24214	2.95	312	
5	1.23812	1.2349	3.22	340	
6	1.23101	1.22783	3.18	336	
7	1.23611	1.23332	2.79	295	
8	1.2325	1.23004	2.46	260	
9	1.2377	1.23477	2.93	310	
10	1.23025	1.22778	2.47	261	
11	1.23399	1.23127	2.72	288	
12	1.23559	1.23402	1.57	166	
13	1.24606	1.24383	2.23	236	
14	1.22845	1.22679	1.66	175	
15	1.22735	1.22463	2.72	288	
control 1	1.22237	1.22268	-0.31	54	std dev
control 2	1.24285	1.24294	-0.09	14	std error
control 3	1.2317	1.23221	-0.51		
control 4	1.23331	1.23383	-0.52		
control 5	1.23434	1.23475	-0.41		
		control adjustment	-0.368	mg VS	
		average biomass per slide	2.55	mg VS	
		biomass per slide (ajd.)	2.91	mg VS	
		surface area of each slide	28	cm^3	
		wetted surface area of reactor	2960	cm^3	
		reactor biomass	308.05	mg VS	

Table C.6 Acetate and VS data for 3.33 µg/L each antibitoic (2)

3-Oct
3.33 ug/L each antibiotic

	Parameters	areal	
μ_{max} =	1.38	0.13	see units below
K_s =	0.0040	mg C/L	
R^2 =	0.925		
VS =	275	mg	
area =	2960	cm^3	

S (mg C/L)	acetate substrate utilization rate (mg C/mg VS/day)	areal substrate utilization rate (mg C/cm^2/day)
0.016	1.044	0.097
0.018	1.044	0.097
0.031	1.042	0.097
0.034	1.390	0.129
0.037	1.390	0.129
0.038	1.390	0.129
1.862	1.420	0.132
2.056	1.387	0.129
2.108	1.377	0.128
4.492	1.310	0.122
4.795	1.257	0.117

slide	dried weight (g)	volatilized weight (g)	delta (mg)	mg VS	
1	1.22492	1.22264	2.28	241	
2	1.24428	1.24103	3.25	344	
3	1.23762	1.2352	2.42	256	
4	1.23639	1.23378	2.61	276	
5	1.24238	1.23999	2.39	253	
6	1.2416	1.23913	2.47	261	
7	1.24612	1.24407	2.05	217	
8	1.2442	1.24274	1.46	154	
9	1.24681	1.24382	2.99	316	
10	1.23433	1.23139	2.94	311	
11	1.22484	1.22243	2.41	255	
12	1.2366	1.23387	2.73	289	
13	1.24473	1.24158	3.15	333	
14	1.2486	1.24608	2.52	266	
15	1.23697	1.23463	2.34	247	
control 1	1.23598	1.23597	0.01	48	std dev
control 2	1.23642	1.23679	-0.37	12	std error
control 3	1.22212	1.22212	0		
control 4	1.23055	1.23046	0.09		
		control adjustment	-0.0675	mg VS	
		average biomass per slide	2.53	mg VS	
		biomass per slide (ajd.)	2.60	mg VS	
		surface area of each slide	28	cm^3	
		wetted surface area of reactor	2960	cm^3	
		reactor biomass	275.02	mg VS	

Table C.7 Acetate and VS data for 3.33 µg/L CIP and 0.33 µg/L ERY and SMX (1)

21-Mar

3.33 ug/L CIP, and 0.33 ug/L ERY and SMX

	Parameters	areal	
μ_{max} =	0.93	0.12	see units below
K_s =	0.0026	mg C/L	
R^2 =	0.952		
VS =	390	mg	
area =	2960	cm^3	

S (mg C/L)	acetate substrate utilization rate (mg C/mg VS/day)	acetate substrate utilization rate (mg C/cm2/day)
0.010	0.736	0.097
0.010	0.736	0.097
0.010	0.736	0.097
0.251	0.953	0.126
0.273	0.950	0.125
0.285	0.948	0.125
1.920	0.993	0.131
1.920	0.993	0.131
2.172	0.962	0.127
4.841	0.880	0.116
5.329	0.820	0.108
5.369	0.815	0.107

slide	dried weight (g)	volatilized weight (g)	delta (mg)	mg VS	
1	1.23028	1.22666	3.62	383	
2	1.23611	1.23228	3.83	405	
3	1.22766	1.22355	4.11	434	
4	1.23956	1.23624	3.32	351	
5	1.24916	1.24633	2.83	299	
6	1.24216	1.23751	4.65	492	
7	1.22213	1.21892	3.21	339	
8	1.23414	1.22955	4.59	485	
9	1.23299	1.22916	3.83	405	
10	1.23256	1.22886	3.7	391	
11	1.23902	1.23516	3.86	408	
12	1.24652	1.24306	3.46	366	
13	1.2437	1.24021	3.49	369	
14	1.24099	1.23775	3.24	343	
15	1.23603	1.23255	3.48	368	
control 1	1.22482	1.2241	0.72	52	std dev
control 2	1.2385	1.23892	-0.42	10	std error
control 3	1.2321	1.23215	-0.05		
control 4	1.2366	1.2369	-0.3		
		control adjustment	-0.0125	mg VS	
		average biomass per slide	3.68	mg VS	
		biomass per slide (ajd.)	3.69	mg VS	
		surface area of each slide	28	cm^3	
		wetted surface area of reactor	2960	cm^3	
		reactor biomass	390.49	mg VS	

Table C.8 Acetate and VS data for 3.33 µg/L CIP and 0.33 µg/L ERY and SMX (2)

24-Nov

3.33 ug/L CIP, and 0.33 ug/L ERY and SMX

	Parameters	areal		
μ_{max} =	0.98		0.14	see units below
K_s =	0.0010	mg C/L		
R^2 =	0.982			
VS =	434	mg		
area =	2960	cm^3		

S (mg C/L)	acetate substrate utilization rate (mg C/mg VS/day)	acetate substrate utilization rate (mg C/cm2/day)
0.010	0.884	0.130
0.011	0.884	0.130
0.469	1.054	0.155
0.603	1.040	0.152
3.706	0.918	0.135
3.723	0.916	0.134

slide	dried weight (g)	volatilized weight (g)	delta (mg)	mg VS	
1	1.24119	1.23726	3.93	415	
2	1.23703	1.23254	4.49	475	
3	1.2361	1.23259	3.51	371	
4	1.23485	1.23106	3.79	401	
5	1.23483	1.2307	4.13	437	
6	1.23123	1.22704	4.19	443	
7	1.23243	1.2286	3.83	405	
8	1.23394	1.23002	3.92	414	
9	1.23822	1.23398	4.24	448	
10	1.23443	1.23042	4.01	424	
11					
12	1.22424	1.21998	4.26	450	
13	1.23493	1.23062	4.31	456	
14	1.23128	1.22696	4.32	457	
15	1.24471	1.24063	4.08	431	
control 1	1.22687	1.22698	-0.11	27	stddev
control 2	1.22521	1.22534	-0.13	7	std error
control 3	1.2282	1.22824	-0.04		
control 4	1.22129	1.22114	0.15		
		control adjustment	-0.0325	mg VS	
		average biomass per slide	4.07	mg VS	
		biomass per slide (ajd.)	4.10	mg VS	
		surface area of each slide	28	cm^3	
		wetted surface area of reactor	2960	cm^3	
		reactor biomass	433.92	mg VS	

Table C.9 Acetate and VS data for 3.33 µg/L ERY and 0.33 µg/L CIP and SMX

20-May
3.33 ug/L ERY, and 0.33 ug/L CIP and SMX

Parameters		areal	
μ_{max} =	1.69	0.12	see units below
K_s =	0.0192	mg C/L	
R^2 =	0.908		
VS =	217	mg	
area =	2960	cm^3	

S (mg C/L)	acetate substrate utilization rate (mg C/mg VS/day)	acetate substrate utilization rate (mg C/cm2/day)
0.102	1.305	0.096
0.106	1.304	0.096
0.106	1.304	0.096
0.186	1.730	0.127
0.187	1.729	0.127
0.188	1.729	0.127
1.993	1.772	0.130
2.042	1.761	0.129
2.164	1.734	0.127
4.800	1.594	0.117
5.146	1.517	0.111
5.186	1.508	0.111

slide	dried weight (g)	volatilized weight (g)	delta (mg)	mg VS	
1	1.23972	1.23802	1.7	180	
2	1.24173	1.23949	2.24	237	
3	1.23186	1.23046	1.4	148	
4	1.24159	1.23915	2.44	258	
5	1.24747	1.24576	1.71	181	
6	1.24751	1.24511	2.4	254	
7	1.23949	1.23759	1.9	201	
8	1.23986	1.23794	1.92	203	
9	1.24973	1.24757	2.16	228	
10	1.24164	1.23983	1.81	191	
11	1.24469	1.24276	1.93	204	
12	1.24437	1.24274	1.63	172	
13	1.24098	1.23849	2.49	263	
14	1.24556	1.24351	2.05	217	
15	1.2474	1.24587	1.53	162	
control 1	1.2228	1.22314	-0.34	36	stddev
control 2	1.23805	1.23803	0.02	7	std error
control 3	1.24047	1.24047	0		
control 4	1.24013	1.2402	-0.07		

control adjustment	-0.0975	mg VS
average biomass per slide	1.95	mg VS
biomass per slide (ajd.)	2.05	mg VS
surface area of each slide	28	cm^3
wetted surface area of reactor	2960	cm^3
reactor biomass	216.87	mg VS

Table C.10 Acetate and VS data for 3.33 µg/L SMX and 0.33 µg/L CIP and ERY

17-Apr
3.33 ug/L SMX, and 0.33 ug/L CIP and ERY

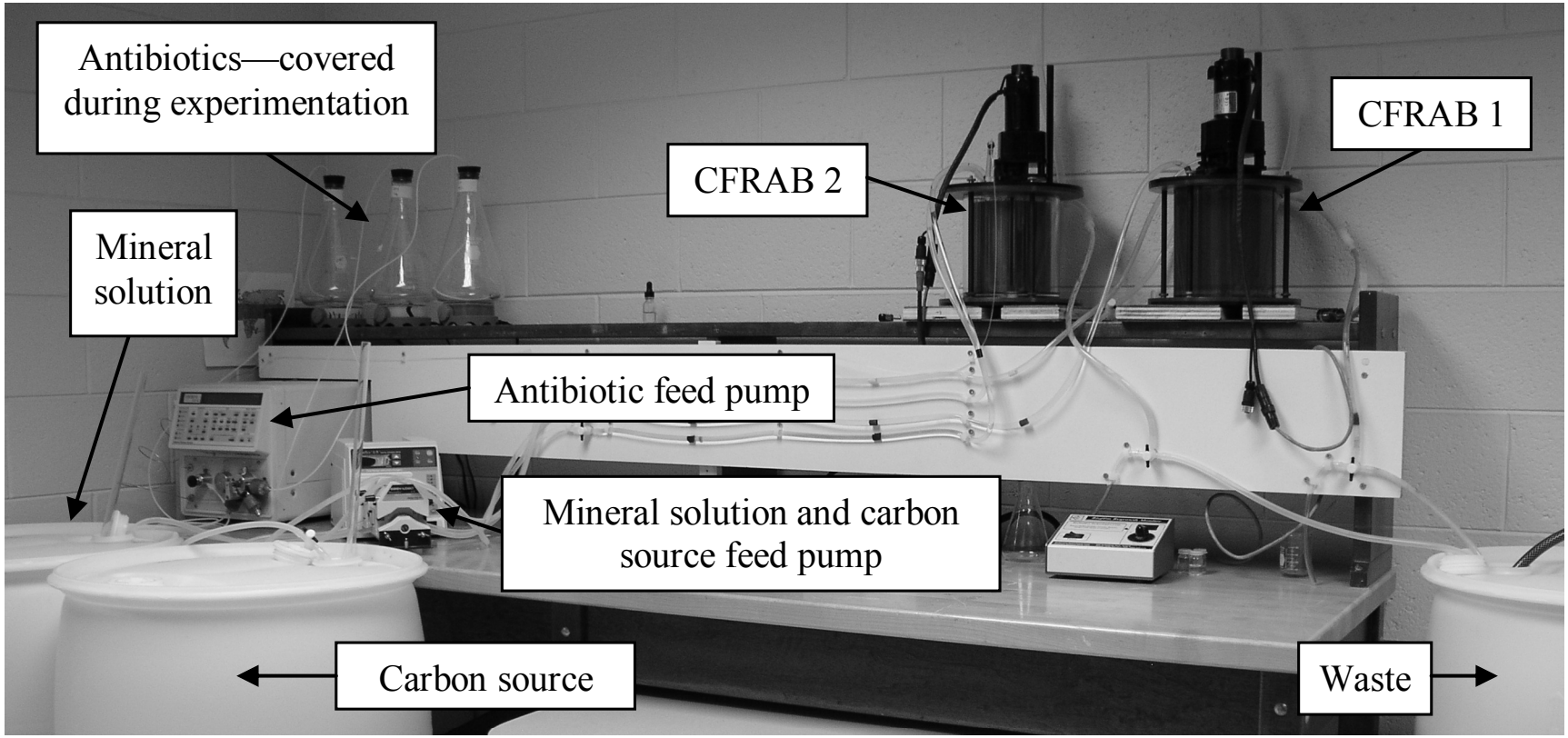
Parameters	areal	
μ_{max} = 1.80	0.15	see units below
K_s = 0.0162	mg C/L	
R^2 = 0.934		
VS = 252	mg	
area = 2960	cm^3	

S (mg C/L)	acetate substrate utilization rate (mg C/mg VS/day)	acetate substrate utilization rate (mg C/cm^2/day)
0.040	1.135	0.097
0.041	1.135	0.097
0.046	1.515	0.129
0.047	1.514	0.129
0.049	1.133	0.096
0.050	1.514	0.129
0.542	1.801	0.153
0.609	1.788	0.152
0.662	1.778	0.151
2.713	1.768	0.151
2.850	1.742	0.148
2.954	1.722	0.147

slide	dried weight (g)	volatilized weight (g)	delta (mg)	mg VS	
1	1.24821	1.24653	1.68	178	
2	1.25083	1.24879	2.04	216	
3	1.2445	1.24189	2.61	276	
4	1.25154	1.24865	2.89	306	
5	1.25173	1.24891	2.82	298	
6	1.23996	1.23865	1.31	138	
7	1.23566	1.23328	2.38	252	
8	1.25021	1.24759	2.62	277	
9	1.2398	1.23745	2.35	248	
10	1.24665	1.24426	2.39	253	
11	1.24292	1.24075	2.17	229	
12	1.23861	1.23664	1.97	208	
13	1.23126	1.22853	2.73	289	
14	1.24613	1.24375	2.38	252	
15	1.24232	1.23943	2.89	306	
control 1	1.23459	1.23459	0	48	stddev
control 2	1.24606	1.24606	0	12	std error
control 3	1.24244	1.24233	0.11		
control 4	1.25225	1.25253	-0.28		
control 5	1.24312	1.24313	-0.01		

control adjustment	-0.036	mg VS
average biomass per slide	2.35	mg VS
biomass per slide (ajd.)	2.38	mg VS
surface area of each slide	28	cm^3
wetted surface area of reactor	2960	cm^3
reactor biomass	252.09	mg VS

Appendix D. Labeled Photograph of Experimental Apparatus used for Sorption Experiments (CFRAB 2 not used)



Appendix E. Further detail on antibiotic sampling and analysis

Sample Collection and Filtration

Immediately preceding the experimental run, effluent was collected for an effluent blank and two effluent standard concentrations (spiked to a known concentration of each antibiotic to be used in the run). These samples were used to ensure that no residual antibiotics from previous runs remain and to determine percent recoveries for antibiotics and internal standards.

For each experimental run, one liter samples were collected every 30 minutes for the first six hours of run-time, and then collected with decreasing frequency thereafter. One liter samples were collected for sample concentration (via solid phase extraction) prior to analysis. Due to the light-sensitivity of the antibiotics, laboratory lighting was reduced or eliminated throughout the run. To further minimize photodegradation, antibiotic feed reservoirs were covered with aluminum foil, and effluent samples were collected in 1-L amber bottles.

Collected samples were filtered using 0.45 μm glass fiber filters (Millipore, Billerica, MA, USA). For each sample, the filter was cleaned with 5 mL HPLC-grade water, then primed with 5 mL MeOH followed by 5 mL HPLC-grade water before the sample was drawn through the filter under vacuum. Throughout sample filtration, aluminum foil covered the flask and funnel in order to minimize photodegradation. Following filtration, samples were stored for no more than two days in 1-L amber bottles at 4°C prior to subsequent processing.

Solid Phase Extraction

Following filtration and storage, samples were concentrated by solid phase extraction (SPE). Because of the low antibiotic concentrations, analysis was improved by concentrating all 1000 mL samples, effluent standards, and the effluent blank to 1 mL. SPE was achieved using the Visiprep solid phase extraction vacuum manifold, drying attachment, large volume samplers, and disposable liners (Supelco, Buchs, Switzerland) and 200 mg Oasis hydrophilic-lipophilic-balanced (HLB) water-wettable copolymer cartridges (Waters, Milford, MA, USA). The HLB cartridges were primed with 6 mL 100% MeOH and 6 mL HPLC-grade water before samples were drawn under vacuum through the large volume samplers and SPE cartridges. Hydrophobic antibiotics are retained on the cartridges. Each HLB cartridge was washed with 1 mL of 5% MeOH in HPLC-grade water to remove unwanted compounds, followed with two separate 3 mL washes of 100% MeOH to elute antibiotics into 15 mL centrifuge tubes (Fisher Scientific, Pittsburgh, PA, USA). The samples were placed in a water bath (55 °C) and concentrated to 1 mL using nitrogen gas to increase the rate of evaporation. Sample exposure to light was minimized with shielding and decreased ambient light levels during processing.

After extraction, samples were transferred to amber autosampler vials (National Scientific, Rockwood, TN, USA). Vials were stored at 4 °C for no more than 2 days prior to analysis. During sample collection, non-extracted standards and blanks were

made in HPLC-grade water, transferred to amber automatic sampling vials (National Scientific 1 mL with snap caps), and stored at 4 °C until analysis.

Internal Standards

Internal standards were used for quantification of samples (Gobel *et al.* 2004; McArdell *et al.* 2003), and to account for losses in sample preparation (e.g., filtration and SPE) and during quantification via Liquid Chromatography/Mass Spectrometry (LC/MS). Surrogate standards were selected for each of the studied antibiotics. Sulfamerazine, Clinafloxacin, and Oleandomycin were selected as internal standards for Sulfamethoxazole, Ciprofloxacin, and Erythromycin, respectively.

Sulfamerazine (SMR) has been shown to be an appropriate internal standard for Sulfamethoxazole (SMX) in similar work (Gobel *et al.* 2004; Renew and Huang 2004). These two sulfonamide antibiotics have very similar chemical structures with similar ionization constants and mass to charge ratios. Oleandomycin (OLE) has been used as a surrogate standard for the quantification of macrolides (including ERY) in surface water (Scribner *et al.* 2003, McArdell *et al.* 2003). ERY and OLE both include the macrolide ring, and other similar functional groups. Clinafloxacin (CLN) is structurally comparable to ciprofloxacin (CIP) and exhibits similar chemical properties (Zabniski *et al.* 1995). Standard solutions, effluent samples, and blanks were all spiked with internal standards at 1.0 µg/L each of CLN, OLE, and SMR before filtration, enrichment and analysis.

LC/MS Analysis

Samples, standards, and blanks were analyzed for antibiotics using liquid chromatography-mass spectrometer (LC-MS) (Agilent 1100 series) with auto sampler and diode array, and positive ion electrospray (ESI(+)) detection. Preliminary work was done to optimize the LC-MS procedure for the antibiotics and the internal standards. The mobile phase was fed at 0.3 mL/min and consisted 40% of 0.3% (v/v) formic acid in acetonitrile and 60% of 0.3% (v/v) formic acid in HPLC-grade water. A Luna 5 micron C18(2) 100 angstrom 150 x 3 mm HPLC column (Phenomenex, Torrence, CA, USA) was controlled at 25°C during LC-MS. The mass spectra detector (MSD) was set to selected ion monitoring (SIM) mode for tailored data extraction in the range of mass-to-charge ratios of the antibiotics and internal standards.

Duplicate injections (10 µL each) of blanks, standards, and samples were fed from the autosampler. Each injection was monitored for a run time of 9 minutes, with a one minute turnaround time between runs. For each injection run, ions were extracted and peaks integrated to quantify the instrument response for each antibiotic and internal standard. Preliminary work was conducted to determine the characteristic response time for peaks associated with each antibiotic and internal standard for the mobile phase and column procedure.

Quantification

Antibiotic concentrations were calculated using an internal standards calibration method and a response factor, then adjusted to account for analyte recovery during sample

preparation procedures. For each antibiotic, x, and the associated internal standard, IS, concentrations [x] and [IS] were known in non-extracted standards and LC-MS instrument responses A_x and A_{IS} were measured. The response factor, F, was then calculated from the relationship

$$F = \frac{A_{x_{Non-extracted Std}}[IS]}{A_{IS_{non-extracted}}[x]}.$$

The responses to x and IS are proportional in all samples because of their similar chemical character; the response factor is the proportionality constant. The average response factors for SMX-SMR, ERY-OLE, and CIP-CLN were 1.01, 0.62, and 0.77, respectively.

After determining *F* for antibiotic-IS pairs, the measured concentration of the antibiotic ($[x]_{sample}$) was calculated for all samples and blanks from the instrument responses and the known concentration of internal standard.

$$[x]_{sample_{measured}} = \frac{A_{x_{sample}}[IS]}{F \times A_{IS_{sample}}}$$

where *F* and [IS] are known and $A_{x_{sample}}$ and $A_{IS_{sample}}$ are found from as a result of LC-MS analysis. The measured antibiotic concentration does not account for the effects of sample preparation. For each antibiotic, $[x]_{measured}$ in the extracted standard was used to find %Recovery.

$$\%\text{Recovery} = \frac{[x]_{measured} - [x]_{baseline}}{[x]_{actual}} * 100\%$$

where $[x]_{baseline}$ is the concentration of antibiotic x in the effluent blank and $[x]_{actual}$ is the concentration of antibiotic in the extracted standard before extraction. The average percent recoveries for SMX, ERY, and CIP were 107, 95, and 155%, respectively. These recoveries are similar to values reported by others for antibiotic quantification via comparable methods. Lindsay *et al.* (1991) reported recoveries ranging from 84 - 130% with a distilled water matrix. Gobel *et al.* (1994) reported recoveries ranging from 18 - 127% for samples of tertiary effluent from a wastewater treatment plant. Renew and Haung (2004) reported recoveries ranging from 37 - 129% for wastewater treatment plant effluent. Percent recovery was used to adjust for the effects of SPE and filtering, and calculate actual antibiotic concentrations in the effluent:

$$[x]_{sample_{actual}} = \frac{[x]_{sample_{measured}} - [x_{baseline}]}{\%R}.$$

Relative standard deviations (RSDs) for the internal standards have been calculated for each run: the RSD for oleandomycin ranged from 11 to 21%, the RSD for sulfamerazine ranged from 10 to 22%, and the RSD for clinafloxacin ranged from 13 to 22%. These RSDs are comparable to values reported by others for antibiotic quantification via comparable methods. Lindsey *et al.* (1991) reported RSDs of 9 – 17% for samples in distilled water. RSDs of less than 15% were reported by Gobel *et al.* (1994) for samples collected from tertiary wastewater treatment. McCardell *et al.* (2003) reported RSDs of 5 – 13% for wastewater treatment effluent

REFERENCES

Göbel, A.; McArdell, C.S.; Suter, M.J-F.; Giger, W. Trace determination of macrolide and sulfonamide antimicrobials, a human sulfonamide metabolite, and trimethoprim in wastewater using liquid chromatography coupled to electrospray tandem mass spectrometry. Analytical Chemistry, 2004, 76:16:4756

Lindsey, M.E.; Meyer, M.; Thurman, E.M. Analysis of trace levels of sulfonamide and tetracycline antimicrobials in groundwater and surface water using solid-phase extraction and liquid chromatography/mass spectrometry. Analytical Chemistry, 2001. 73(19):4640

McArdell, C.S.; Molnar, E.; Suter, M.J.; Giger, W. Occurrence and fate of macrolide antibiotics in wastewater treatment plants and in the Glatt Valley watershed, Switzerland. Environmental Science and Technology, 2003. 37(24): 5479

Renew, J, and Huang, C.H. Simultaneous determination of fluoroquinolone, sulfonamide, and trimethoprim antibiotics in wastewater using tandem solid phase extraction and liquid chromatography-electrospray mass spectrometry." Journal of Chromatography A, 2004, 1042: 13.

Scribner, E.A.; Battaglin, W.A.; Dietze, J.E.; Thurman, E.M.. Reconnaissance data for glyphosphate, other selected herbicides, their degradation products, and antibiotics in 51 streams in nine midwestern states, 2002. U.S. Geological Survey: Lawrence, Kansas,

Zabinski, R.A.; Walker, K.J.; Larsson, A.J.; Moody, J.A.; Kaatz, G.W.; Rotshafer, J.C. Effect of aerobic and anaerobic environments on antistaphylococcal activities of five fluoroquinolones. Antimicrobial Agents and Chemotherapy, 1995, 39:2:507

Notes on Antibiotic Sampling, Preparation, and Quantification Processes

Step 1. Preparation for an Experimental Run

Overview: Before collecting samples of antibiotics for the purpose of quantification, several things must be established. The individual must make sure that all of the necessary preparatory steps have been taken.

1. Filtration hardware
    - At least 12 1-L amber bottles (more than enough for a full run)
    - Strong Vacuum Pump (able to create vacuum of up to 15" Hg) with Regulatory Valve and Gauge attachment
    - ENVI Disk Cleaning materials (0.2 µm fiber filters, funnel, collector, 25mm X 250mm test tube, vacuum water trap)
    - 1-L of MeOH (Methanol)
    - Type 1 water for calibration standards and periodic cleaning.
    - Ample Deionized water to be used for cleaning (if Type 1 not available).
    - Miscellaneous glassware (three or four 100-mL beakers should be enough) to be used for holding MeOH, water and other solutions.
    - 5000 µL pipette, 1000 µL pipette with at least half a box of tips each.

2. Solid Phase Extraction hardware
    a. Up to 12 Oasis HLB SPE cartridges (for a full run)
    b. Up to 12 large volume samplers (for a full run)
    c. Plastic Disposable Liners to connect the manifold to the SPE cartridges
    d. Supelco Visiprep Vacuum Manifold
    e. Supelco Visidry Drying Attachment
    f. 12 x 15-mL centrifuge glass tubes with snap caps
    g. A lab-grade N_2 tank with attachments to connect to the Visidry attachment
    h. Water Bath capable of 55 °C temperatures.

3. Make sure that there is space in a readily available refrigerator (4 °C) to store 12 1-L amber bottles.

4. LC-MS Quantification
    a. Hope College currently provides us with access to their LC-MS.
        Contact Hope College Chemistry Department
        (Mike Seymour Seymour@hope.edu or Donna Sovasova@hope.edu)
        Science Center Rm. 2057
        35 E. 12th St.
        Holland, Michigan 49422-9000 USA
        Voice: (616) 395-7635
    b. Remember to bring along standards, blanks, and samples.

5. Analysis
    a. Microsoft Excel can be used as a method of tabulating the areas of peaks from chromatograms associated with each antibiotic at a given time.
    b. Use Excel graphs to determine calibration curves from standards ('known' concentrations), which are then used to calculate concentrations in samples ('unknown' concentrations)

Step 2. Collection of Bioreactor Samples

To quantify antibiotics within the bioreactors/columns, 3 types of samples must be taken.

1. **Effluent Blank**: A 1-L sample of the effluent from the bioreactors/columns before the antibiotic has been added is needed to be able to compare with the actual samples. Solid Phase Extraction (SPE) and concentration must be performed to ensure the closest possible comparison with the real samples. Internal standards (at a known concentration – 1 mg/L each after SPE) are also added.

2. **Extracted Calibration Standards**: Take control effluent (for bioreactors, prior to the addition of the antibiotic(s) and for column tests, from the control columns). Inject known concentrations of each antibiotic into this matrix. Normal SPE and concentration must be performed on Standard 2. These standards are used to construct calibration curves.

3. **Samples**: The rest of the 12 1-L total samples are simply collected at a set interval once antibiotics have been injected into the bioreactor/columns. Internal standards must be added to these samples during collection. They then receive basic filtration and SPE before being stored with the standards until quantitation is possible.

Be sure to make/use the sample collection table for each run. This spreadsheet could also leave room for the LC-MS file names/numbers for analysis and other comments.

Step 3: Filtration

Overview: The filtration step is necessary to remove the solids which are present in each sample of CFRAB effluent. Each sample requires the following processes, with a triplicate rinse of DI water of the components required in between.

1. **ENVI-Disk Holder Processes** *(Total Time is about 30 minutes)*

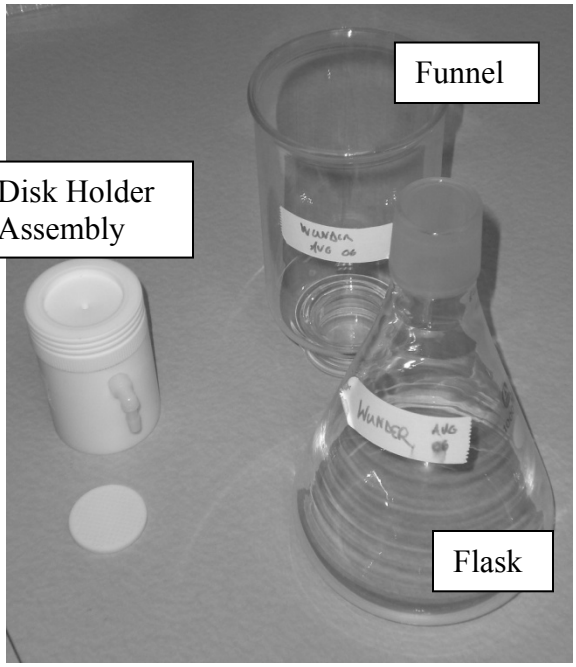


Figure E.1. ENVI Disk Equipment

a. *Funnel and ENVI-Disk Cleaning*:
   i. Remove the disk holder assembly (Figure E.1) from the flask.
   ii. Place a 25mm x 250mm test tube in the flask to collect waste.
   iii. Insert a Millipore 0.2 µm pore fiber filter disk on top of the funnel drain with the patterned side up. Replace the disk holder assembly.
   iv. Clean the funnel and the disk by washing with approximately 5 mL of DI water. Use the larger of the two micropipettes for this step. Allow the water to soak the disk for approximately 30-60 seconds.
   v. Close the needle valve (which is in-line with the vacuum tubing) and turn on the vacuum pump to draw solvent through the disk. Maintain 15" Hg for 5 minutes.
   vi. Release the vacuum by opening the needle valve (with vacuum still on).
   vii. Remove the test tube containing solvent waste.
   viii. Put the disk holder assembly on top of the flask.

b. *ENVI-Disk Conditioning and Sample Addition:*

i. With vacuum pump still on, use the needle valve to throttle vacuum to 2” Hg (plug other end of tubing with your finger).
ii. Unplug vacuum tubing from the filter to let the flask vent completely.
iii. Add 5 mL of Methanol to the glass funnel and let the disk soak for approximately one minute (or until the MeOH begins dripping through the filter) to activate the filter. Do not worry about the materials ending up in the sample collector.
iv. Further condition the filter by adding 5 mL of DI water to the funnel once the Methanol begins dripping through the filter.
v. While the 5 mL of DI is still draining, add the prepared 1-L samples which is stored in a amber bottle (Figure E.2) to the funnel.

Figure E.2. 1-L Amber Bottle

vi. Reconnect the vacuum tubing to the flask and check to make sure pressure is about 2” of Hg.
vii. When the entire sample has passed through the disk, close the needle valve to achieve 15” Hg, leaving the maximum vacuum for 10 minutes to dry.
viii. Store the samples in a cold room (4 °C) until ready for extraction up to 10 days later.

Step 4. Solid Phase Extraction/Sample Concentration

Overview: Sample concentration is needed to raise the concentration of the antibiotics present in the bioreactor effluent to a traceable level. If 1-L sample containers are used and brought to a resulting volume of 1-mL, an increased concentration of 1000X has been accomplished. By carefully adhering to the following steps, successful concentration of the antibiotics within the samples will be accomplished.

1. Sample Concentration *(total time is about 3 hours)*
    a. *Placing the samples on the SPE Cartridge*
        i. Connect the large liquid trap (25 L) between the manifold (Figure E.3) and the vacuum pump.

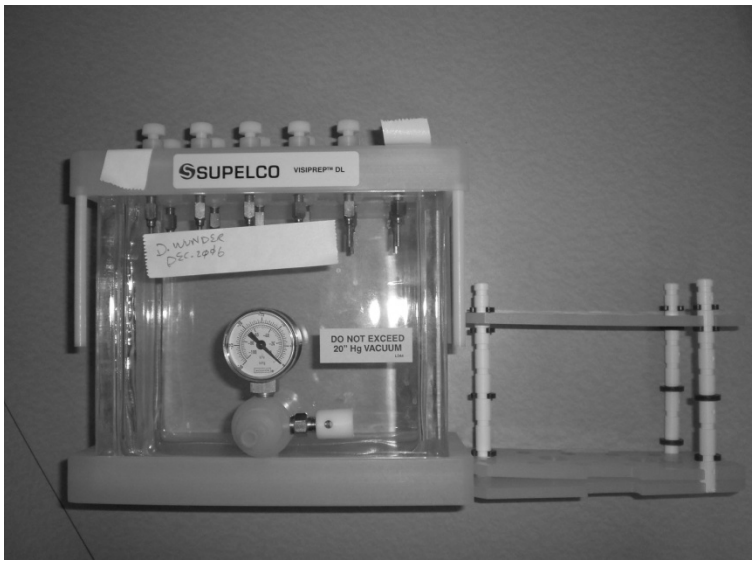


Figure E.3. Vacuum Manifold with Rack

        ii. Make sure the Disposable Liners (shown in Figure E.4) are inserted in all the SPE slots even if they aren't going to be used. Insert liners slowly to avoid breaking or bending. If one bends, do not use it. Put liners in all slots whether or not you are doing 12 samples. Make sure to label each position on the top manifold.

Figure E.4. Disposable Liners (DL's)

iii. Close the knurled valves on top. Look through the bottom of the manifold lid through the hole to see that the slit is closed on each hole (clockwise turns to close). Solutions typically are added to the SPE tubes with the flow control valves closed.
iv. Insert the Waters SPE cartridges into the DL's, twisting them in to ensure a tight fit and that the cartridges face the same way to keep track of how tight or loose they are.
v. Turn the vacuum pump on, with the in-line bleed valve loose.
vi. Add 2 X 3-mL of MeOH (2 X 3 mL means to add 3mL MeOH, open valve, let dry, repeat) to each cartridge with an automatic pipette-injector to activate the cartridge. (dry for 10-15 seconds after each 3 mL)
vii. Grasp the knurled flow valves and make a ¼ turn counterclockwise, to partially open the flow control valve.
viii. With the needle valve in-line with the vacuum pump loosened, tighten the needle valve on the manifold. Leave this manifold valve fully tightened and use the vacuum pump valve as the main throttle. Tighten this in-line needle valve enough to where the volume is emptying dropwise.
ix. Allow the flow to empty contents of each SPE cartridge.
x. Let dry for 10-15 seconds.
xi. Loosen the in-line vacuum bleed valve all the way.
xii. Close the knurled flow valves.
xiii. Add 3-mL of Type-1 water to each cartridge further prepare the cartridges.
xiv. Grasp the knurled flow valves and make a ¼ turn counterclockwise, to partially open the flow control valve.
xv. With the needle valve in-line with the vacuum pump loosened, tighten the needle valve on the manifold. Leave this manifold

valve fully tightened and use the vacuum pump valve as the main throttle. Tighten this in-line needle valve enough to where the volume is emptying dropwise.

xvi. Allow the flow to empty contents of each SPE cartridge.

xvii. Let dry for 10-15 seconds.

xviii. Loosen the in-line vacuum bleed valve all the way.

xix. Close the knurled flow valves.

xx. Add 3-mL of Type-1 water to each cartridge further prepare the cartridges.

xxi. Rinse the outside of the large volume samplers. Connect the large volume samplers to manifold and then stick the metal end of the samplers into the 1-L samples (amber bottles) to be extracted.

xxii. Open the knurled flow valves ¼ turn.

xxiii. Tighten the in-line bleed valve such that the water begins flowing dropwise through the needles.

xxiv. Ramp up the pressure slowly (to avoid spraying) on the in-line bleed valve to 15" Hg. Open knurled valves enough to provide good flow (maybe more than ¼ turn). This process takes 2.5 hours. Be sure to be using 12 samples if at all possible.

xxv. Rinse Type 1 water through each large volume sampler when finished with it. Do this by having a beaker with Type 1 in it and flush the water through the SPE cartridge for a few seconds followed by exposing the sampling side of the sampler to the air until the tube is empty. The sampler can then be put away.

xxvi. Close the knurled valve on each hole that has completed passing the sample through it to keep a good vacuum.

xxvii. Open the in-line vacuum bleed valve all the way when finished with all 12 samples.

b. Eluting the Sample

i. Inject 1-mL of 5% MeOH (in HPLC-grade water) into each SPE cartridge with the flow-control knobs open to elute unwanted materials from the SPE cartridges.

ii. IMPORTANT! Remove the manifold cover and place the collection vessel rack containing the 15 mL glass centrifuge containers (shown in Figure E.5) into the basin. **DO NOT FORGET THIS, else all the samples will be lost.**

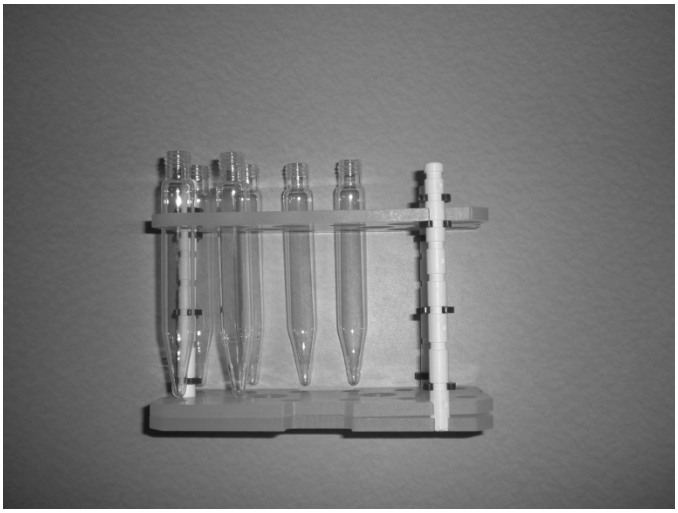


Figure E.5. Manifold Rack with Centrifuge Tubes

iii. Tighten the knurled flow-control valves completely.
iv. Add 2 x 3 mL of MeOH (this means 2 separate elutions of 3-mL of MeOH), then close the vacuum bleed valve and open each knurled flow control valve slowly, just enough to allow the eluate to flow dropwise through the tube **at about 2 in Hg.**
v. Turn on vacuum to 15" Hg after solution has been eluted to make sure that the cartridge is completely empty (approximately 2 minutes).
vi. Open the vacuum bleed valve and then throw away the SPE cartridges.
vii. Keep the Disposable Liners inside their place to connect to the Visidry Attachment.

2. Drying/Evaporating Eluates using the VisiDry Attachment (Total time about 30 minutes)
    a. Place collection vessel rack containing samples in the water bath..
    b. Install Visidry attachment (Figure E.6) onto the vacuum manifold cover. Be sure that the clips on the ends of the unit snap into place beneath cover.
    c. Screw knurled wheels all the way down on plastic rods on support unit. Do not use support rod extensions. Master shut-off valve should face rear.

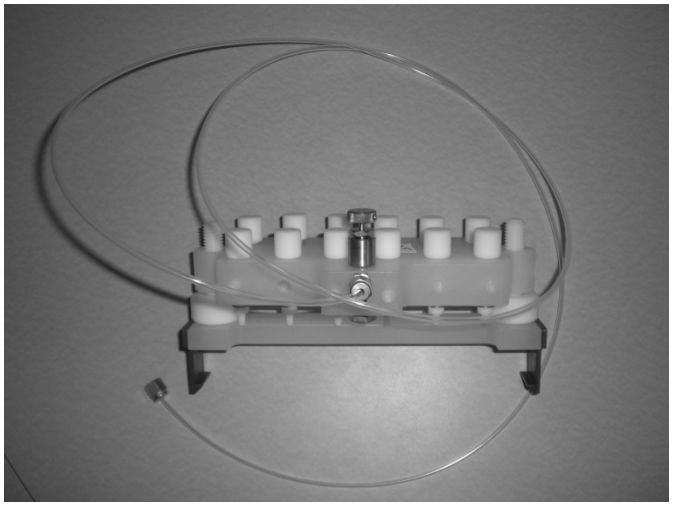


Figure E.6. Visidry Attachment

d. Seat each male luer fitting on drying attachment plate in corresponding female luer fitting on vacuum manifold cover. Do this by pushing down on control knobs and rotating them clockwise.
e. Turn each control knob ¼ turn counterclockwise, then ¼ turn clockwise. This will ensure that each flow control valve on manifold cover turns when corresponding control knob is turned. Once fittings are properly joined, turn all control knobs attached to tubes clockwise to close flow control valves.
f. Be sure bath is set to approximately 55 °C.
g. Set the manifold cover on top of the rack (with the 15-mL centrifuge tubes submerged in the bath up to the sample level) to have direct N_2 access to the samples.
h. Turn knob on master shut-off valve to OFF position (fully clockwise).
i. Using appropriate brass fittings, attach other end of tubing to pressurized N_2 gas source via a pressure regulator with a gauge.
j. Open gas valves on the N_2 cylinder (the large metal one and then the little black one on the regulator) and set pressure to no more than 40 psi with the Increase/Decrease knob.
k. Open master shut-off valve slowly on the Visidry attachment.
l. **CAUTION**: If valves are opened too far too quickly, sample may be lost due to splashing. Carefully open master shut-off valve on drying attachment plate by turning knob counterclockwise about ¼ turn. *Slowly* open individual flow control valves on vacuum manifold cover by pressing down on each control knob and turning it counterclockwise. Adjust rate of gas flow to each collection vessel as needed (i.e. top of samples "dance")..
m. When extracts have been evaporated down to near dryness (~100 µL) turn off gas source. Wait for gas flow to cease. **PREFERRED:** You can also simply dry down to a volume of 1-mL to obtain an increased concentration by a factor of 1000 and not follow step N below.

n. Lift off vacuum manifold cover/drying attachment assembly and remove collection vessel rack from basin.
o. Reconstitute samples with MeOH to 1-mL volume if not already at that volume. Transfer to LC-MS autosampler vials and store in a refrigerator/cold room. A refrigerator with a temperature below at least 4 °C is strongly recommended. Also, do not let these samples sit (even in the refrigerator) more than 10 days before quantification is performed.

Step 5: Preparation of non-extracted standards/blanks

Overview: Non-extracted standards/blanks are analyzed to determine recovery and noise.

1. Determine appropriate concentrations for standards (most likely the same as the extracted calibration standard concentrations).
2. Make solutions at the concentrated concentration (e.g. if the extracted standards are made in the amber bottles at 6, 2, 1, and 0.1 ug/L, they will be concentrated to 6, 2, 1, and 0.1 mg/L during SPE. Therefore, the non-extracted standards should be made up as 6, 2, 1, and 0.1 mg/L).
3. Transfer approximately 1 mL of each solution to an appropriately labeled autosampler vial for LC-MS analysis.
4. NOTE: these solutions can be used to make up the extracted calibration standards (add 1 mL of the "non-extracted standard" solution to an amber bottle and fill to 1 L)

Step 6: Antibiotic Quantification/ LC-MS

Overview: Be sure that you have received permission to begin using the LC-MS equipment at Hope before beginning day-long runs. Don't forget ALL blanks, standards, and samples.

The following conditions were developed to retrieve optimal data. Most (if not all) of the following conditions will be defaulted to once the method "Wunder1" is loaded and the sequence "Wunder" is loaded as well. Simply check each step before a run is performed to make sure this is the case.

1. Getting ready to Run
    a. **If the pumps are not already running**, be sure to click the "On" button which is located in the View→ Run Control screen. Check mobile phase volume levels (see below for more info). Let pump run to acclimate the column while setting up other parameters.
    b. **Load Method** by clicking the Method drop-down and selection "Load Method". Click the down-arrow and select *Wunder1* as the name of the method.
    c. **Load Sequence**.
        i. Set up this as a Sequence by clicking the Sequence → "Sequence Parameters". Select *Wunder* as the sequence to use.
    d. **Check Tune**.
        i. View → MSD Tune (6^{th} Entry)

ii. In the Select Tune File window, click the "Positive Polarity" option under "ATUNES.TUN".
iii. Tune → Check Tune. This will lead the program through a sequence of events which will end up giving the user a printout and a file saved.
iv. NOTE: In May 2009, Prof. Mike Silver shared that the Check tune was not responding at times. The "Full Tune" option requires more time, but may result in a "pass" when a Check Tune returns a "fail"
v. Three hole-punch the printout and put it on the top of the gray 3-ring binder. Also, enter the data of the current run into the other gray Log Book.

e. **View Menu**. The most frequently used portions of the LC-MS software are the first two options under the View drop-down menu. What follows is a brief description of both.
    i. Method and Run Control [First Option in the View Menu]
        1. Pump (Set up Pump)
            a. Can change flow and the mobile Phase breakdown
            b. Make flow = 0.3 mL/min
            c. Make Mobile Phase A = 0.3% Formic Acid in ultrapure water
            d. Make Mobile Phase B = 0.3% Formic Acid in Acetonitrile
            e. Make A:B = 40:60
            f. Injection Volume = 10 μL.
            g. Stop Times typically 8-15 min (varies based on analytes. Watch for late peaks that may interfere with subsequent injections)
            h. Typical pressure during runs: 20-50 bar.
        2. Column Thermostat Method
            a. Leave as it is (25 °C).
        3. MSD
            a. Set up range of ions for the Mass Spec to search for.
            b. Set up MSD stop time "as pump"
            c. With current antibiotics, switch to SIM mode instead of scan mode. Otherwise, the results will be nearly untraceable for Ciprofloxacin. Enter each of the ion wavelengths to search for as shown in Table E.1.

Table E.1: Typical M/Z ratios for Analyte Antibiotics and Internal Standards

Analyte	Precursor Ion [M+H]+ (m/z)	Approximate Peak Time*
Sulfamethoxazole (SMX)	254	6.9 min
Sulfamerazine (SMR) - IS	265	6.4 min
Ciprofloxacin (CIP)	332	3.8 min
Clinafloxacin (CLN) - IS	366	3.9 min
(Anhyro)Erythromycin (ERY)	716.5 [Anhydro 734]	3.9-4.2 min^{+}
(Anhydro) Oleandomycin (OLE) - IS	688.4 [Anhydro 544.3]	3.8-4.1 min

*Peak times may vary slightly between analytical runs (+/- 0.2 min?), but should be less variable within an LC-MS sequence

$^{+}$ERY (and OLE, to a lesser extent) show peak-splitting on many runs. Take peaks in the time range with similar heights, and find the total area as the sum of those areas (e.g., ERY is 716.5 and ERY·H_2O is 734)

NOTE: Confirming ions have been neglected since they contributed insignificant mass in early trials.

4. Diode Array Detector
    a. Not applicable to MS application, only the range that the UV spectra are scanned. It is appropriate to leave this setting to contain a large range of spectra for the Diode Array to look for.

ii. Data Analysis [Second Option in the View Menu]
1. TIC (Default graph on bottom of screen)
    a. Shows net ions in MS form
    b. X-axis = m/z ratio
    c. Y-axis = Total Counts
    d. Click on Parabola with mouse (after click the arrow) at a particular time to see ion breakdown
2. Any other ions which have been selected will be shown on separate graphs if selected to be shown. This must be done before integrating to ensure each ion is separate.

f. **Prepare the proper mobile phases for your operation**. Ensure that these mobile phases are connected and selected under the "Pump" section of the View→Method and Run Control Segment.

g. Refilling mobile phase bottles. Before leaving the LC-MS to run for any significant amount of time, check the bottles to ensure you have adequate mobile phase volume for the run.

To make Mobile Phase A: Use ultra-pure water from the Hope Chemistry department water filter (found in the room near the staircase; ask one of the professors to unlock the door). For 0.3% Formic Acid, add 1.5 mL from the bottle located in the LC-MS room to 500 mL in a clean 1-L bottle.

To make Mobile Phase B: Same as Mobile Phase A, except use acetonitrile from the LC-MS room (rather than water).
IMPORTANT: Turn off the pump (after one sample run time is complete and before the next injection), and use a clean funnel to add the new mobile phase to the bottles on top of the LC-MS. Restart the pump and begin the sampling procedure where you left off. Adjust the "Bottles Filling" to reflect the added volume.

h. **If you have to make up and connect *new* mobile phases**, the lines must be purged of the previous mobile phases:
    i. Open the black valve on the front of the LC equipment to send the stream to waste.
    ii. Set Mobile Phase A at 100% at a high flow rate (4 mL/min) in the Pump section of the first screen. Allow this to flow for a couple minutes, and then expose the end of the sampler to air.
    iii. Then place the sampler into the new Mobile Phase A.
    iv. Repeat this process with the sampling tubing which is connected to Mobile Phase A.
    v. Reset the flow rate to 0.3 mL/min.

i. Installing a new column
    i. Take off the column housing.
    ii. Connect the upstream side of the column to the tube labeled "# 3", and connect the downstream side of the column to "#2".
    iii. Be sure to run plenty of mobile phase through the new column to rinse out any stuck matter inside of the column.

2. Run Procedures
    a. **Be sure that you are operating the MSD in SIM mode if at all possible**. Also, be sure that the polarity is "positive". Check to make sure that the right ions are selected in the list of ions looked for.
    b. **Stop Time**. In Pump→Settings, be sure to set the correct "Stop Time". 9 minutes is usually adequate for a mix of the three previously used antibiotics. (This can be adjusted if noise is observed at the end of any runs)
    c. **Be sure to start a new file tree before performing any runs by saving the current settings.**
        i. Click on the image of the single vial if doing one sample at a time, and then click "Sample Info" to access the window which allows you to change the name. For multiple injections, click "Sequence" → "Sequence Parameters" to change the name.
        ii. Names are typically saved in the 2 digit month, 2 digit day, and 2 digit year format (e.g. 07250701.D would be the first sample taken on July 25, 2007).
        iii. Change the "Prefix" box to the date and the "Counter" box to 01 if it's the first run of the day. Otherwise, setting the counter to 10 is a safe bet to make sure you don't erase over the first runs of the day. Also, be sure to edit the "Comments" box to briefly describe what data is being analysed.

d. **Multiple Injections**. Click the "multiple vial" image in the upper left hand corner of the View→ Run Control screen.
e. **To edit the samples and their conditions**. Click Sequence→ Sequence Table and start editing appropriately. Check against information below to make sure everything is up to snuff.
f. **Start Sequence**.
    i. Click on the Green button which says "Start" to begin a run.

3. During a Run
    a. **Continually check the "Data Analysis"** section of the "View" drop down to make sure that peaks are coming out at the times that they should be.
        i. Click File→Snapshot
    b. **Ciprofloxacin** should come out at 3.7 mins, Clinafloxacin sometime around this.
    c. **Erythromycin** should come out at 3.8 mins, Oleandomycin as well.
    d. **Sulfamethoxazole** should come out at 7.0 mins, Sulfamerazine also.
4. Troubleshooting
    a. If something looks incorrect, feel free to click the "Stop" button to immediately stop the run. No harm will come to the sample or to the data, the machine will simply quit performing the requested run. Note that any compounds already injected into the column will continue to come off after their typical chromatographic time from injection.
    b. If peaks are coming off of the column much earlier than expected in the chromatogram, and are relatively wide, this peak could be a delayed response from a previous run. Typically, earlier peaks are narrow and later peaks are wide.
    c. If you notice a **sinusoidal rhythm** to the MSD output, a common fix can sometimes correct this error:
        i. First open the black valve on the LC
        ii. Turn the "Pump" onto a higher flow rate such as 4 mL/min
        iii. Change the Mobile Phase to 100 % of a liquid which hasn't been used in awhile.
        iv. Allow the machine to run for several minutes.
        v. Change the LC-MS back to original conditions, but wait to close the black valve until the flow rate has returned to the original quantity.
        vi. The signal should return to giving off a steadier instrument response. If it does not, repeat this process.
    d. If the main graph under the first option of the View drop-down menu is unreadable, then some changes must be made under "Online Plot Options".
        i. The "Change" button adjusts the selected graph. Set the maximum value extremely high to be able to see everything available.
        ii. The "Adjust" button sets the display to 10% of full scale. Sometimes this option helps and sometimes it does not.
        iii. The "Balance" button sets the UV detector such that the sample is balanced with reference to the signal.

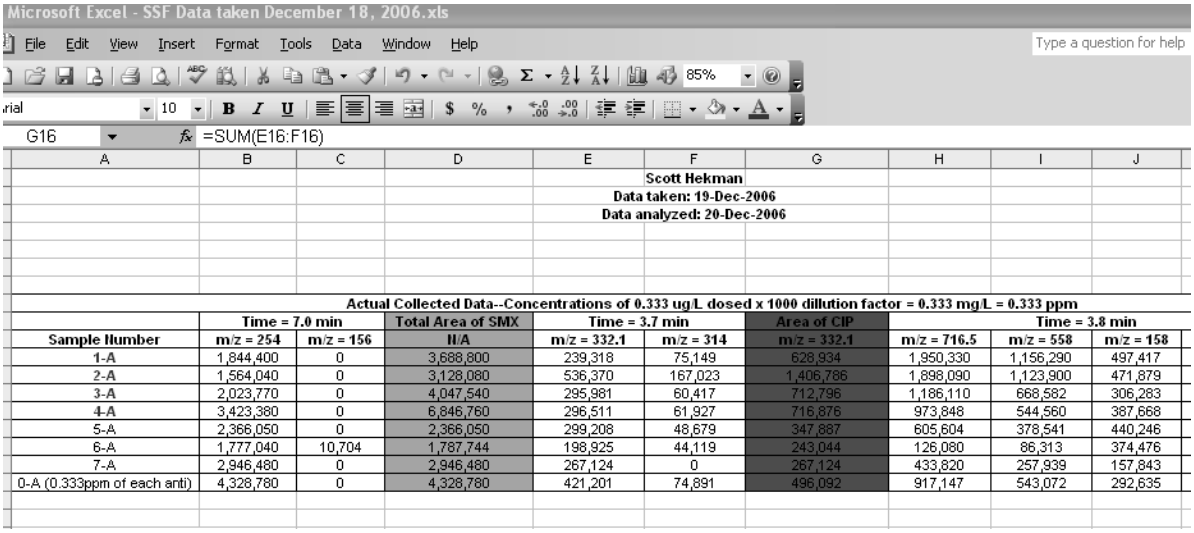
5. Quantitation
    a. After the run is completed, you can integrate the peaks to get an area of counts from which concentration can be found. Sample concentration is directly proportional to chromatograph area.
        i. **To integrate**:
            1. Go into the View drop-down and click "data analysis".
            2. Next, click the File drop-down and select "Load Signal". Here you will have to remember the name of the file you want to load. Only one file may be loaded at a time, so each file must be called up and printed individually.
            3. Add a graph of each ion you want to have integrated by clicking the File drop-down and then clicking "Extract Ions". You can then type-in the ion mass/charge ratio of the ions you are looking for.
            4. To integrate the peaks of the instrument response, click the Integration drop-down and then click "Auto Integrate". This process will take a minute to perform.
        ii. **To print and save the report to a flash drive**:
            1. Begin by simply clicking the File drop-down and click "Print Report".
            2. Be sure that the destination is the printer. If you wish to send the data to a file as well (this is usually the case), then make sure to click the "File" checkbox under destination as well. A good filetype to save as is "xls". Also, make sure that you change the Prefix to whatever the date is, an example is "22June".
            3. To retrieve this data later, right click on the Windows "start" menu which is in the bottom left of the screen. Then click "explore". The filepath that the data is saved under is defaulted to C/HPChem/1/DATA/MDS/whatever the run date was.
            4. You then have to click the individual folder and find the filename that you saved it as, an example is "22June01.xls". Be sure to transfer this file to your flash drive, if you brought one.
            5. Record the names you assign to each replicate and sample. Assign these in the order of the run (e.g. 22June01.xls would be the first injection on June 22, and 22June11.xls would be the eleventh injection – most likely the second injection for the 5^{th} vial in the autosampler)
        iii. **To print the ionic makeup of any point in time of the MSD1 chromatogram**, a few steps must be followed:
            1. In the Data Analysis section, mouse over and click on the DNA strand icon.
            2. Scroll to the MSD1.

3. Click the curve with the arrow icon, and then select the time you want to examine. Be sure you then click anywhere inside of the newly opened window.
4. Now click File, Print, Selected Window.

Step 7. Sample Analysis

Overview: After all of the reports of the samples and standards have been printed off from the LC-MS equipment, analysis can be conducted. Microsoft Excel is the only program which must be employed in this step. There are three major steps involved in analyzing data.

1. Gleaning information from the Reports
    a. Take the areas of the peaks reported in each section of the Integration Reports from Hope and enter them in a spreadsheet. If there is more than one ion for a particular antibiotic, be sure to add up each of the areas to come up with the Total Area for each compound. This process is represented in the image below (Figure E.7.).



Scott Hekman
Data taken: 19-Dec-2006
Data analyzed: 20-Dec-2006

Actual Collected Data--Concentrations of 0.333 ug/L dosed x 1000 dillution factor = 0.333 mg/L = 0.333 ppm

	Time = 7.0 min		Total Area of SMX	Time = 3.7 min		Area of CIP	Time = 3.8 min		
Sample Number	m/z = 254	m/z = 156	N/A	m/z = 332.1	m/z = 314	m/z = 332.1	m/z = 716.5	m/z = 558	m/z = 158
1-A	1,844,400	0	3,688,800	239,318	75,149	628,934	1,950,330	1,156,290	497,417
2-A	1,564,040	0	3,128,080	536,370	167,023	1,406,786	1,898,090	1,123,900	471,879
3-A	2,023,770	0	4,047,540	295,981	60,417	712,796	1,186,110	668,582	306,283
4-A	3,423,380	0	6,846,760	296,511	61,927	716,876	973,848	544,560	387,668
5-A	2,366,050	0	2,366,050	299,208	48,679	347,887	605,604	378,541	440,246
6-A	1,777,040	10,704	1,787,744	198,925	44,119	243,044	126,080	86,313	374,476
7-A	2,946,480	0	2,946,480	267,124	0	267,124	433,820	257,939	157,843
0-A (0.333ppm of each anti)	4,328,780	0	4,328,780	421,201	74,891	496,092	917,147	543,072	292,635

Figure E.7. Excel Image showing Tabular Arrangement

2. Internal Standards
    a. Calculating Concentration using Internal Standards:
        i. To best utilize internal standards, the same concentration of internal standards must be present in each blank, sample and standard.
        ii. Antibiotics and their corresponding internal standards must be compared with one another (e.g. Sulfamethoxazole (SMX) must be used in conjunction with the internal standard Sulfamerazine (SMR)). This means that with 3 antibiotics, 3 equations are generated.

3. Creating Calibration Curves
    a. Using the graphing function of Excel, plot the [Analyte Area]/[IS area] as a function of known concentration for all extracted calibration standards as shown in Figure E.8. Use a trendline for each antibiotic to determine the relationship. Be sure to account for the 1000X concentration factor from SPE.

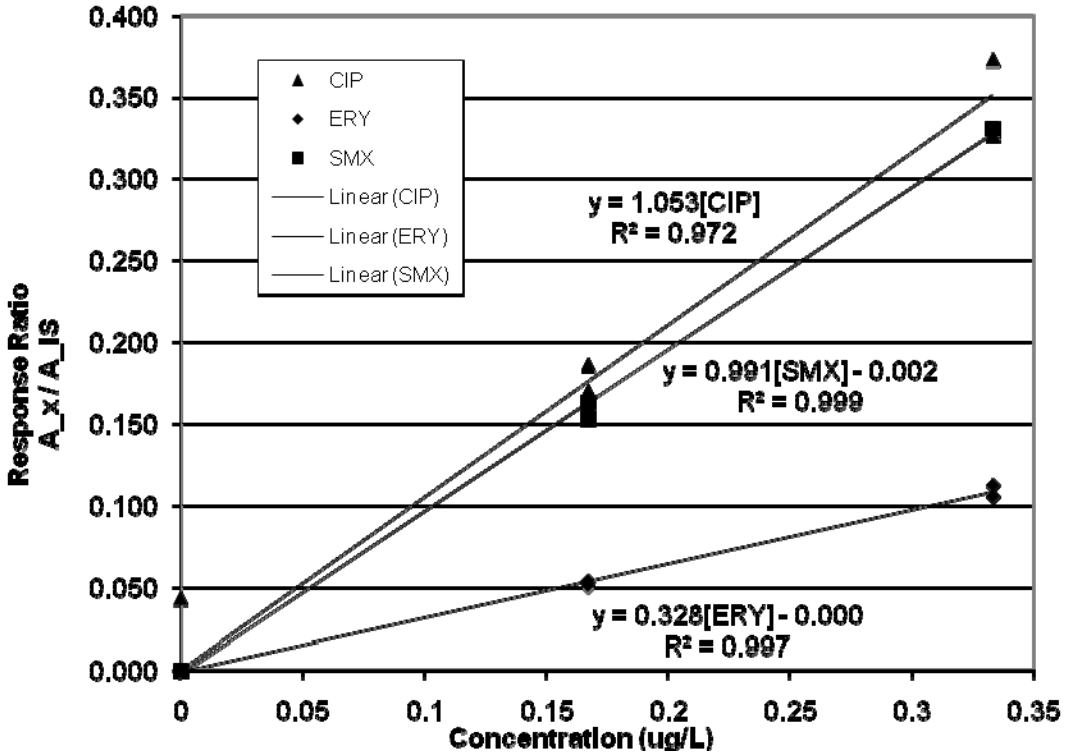


Figure E.8. Example Calibration Curve

    b. These calibration curves are then used to determine the antibiotic concentrations in the samples collected from the bioreactor/columns. The [analyte]/[IS] ratio from the LC-MS data is plugged into this function in order to solve for the analyte (antibiotic) concentration in the sample.
    c. New plots can then be generated to monitor the change in concentration of the antibiotics over time in the bioreactor.

Step 8: Chemical Information and Handling

Name	Function	Handling	Mfg	Part No
Sodium Acetate [$NaCH_3COO$] [s]	Making Acetate Feed	Lab gloves, Lab glasses	Sigma-Aldrich	S2889-250G
Potassium Phosphate Dibasic Trihydrate [K_2HPO_4] [s]	Making Synthetic Water	Lab gloves, Lab glasses	Sigma-Aldrich	P5504-500G
Potassium Phosphate Monobasic [KH_2PO_4] [s]	Making Synthetic Water	Lab gloves, Lab glasses	Sigma-Aldrich	P0662-500G
Sodium Phosphate [Na_2HPO_4] [s]	Making Synthetic Water	Lab gloves, Lab glasses	Sigma-Aldrich	S9763-500G
Magnesium Sulfate [$MgSO_4$] [s]	Making Synthetic Water	Lab gloves, Lab glasses	Sigma-Aldrich	M7506-500G
Iron(III) Chloride Hexahydrate [$FeCl_3*6H_2O$] [s]	Making Synthetic Water	Lab gloves, Lab glasses	Sigma-Aldrich	236489-100G
Ammonium Chloride [NH_4Cl] [s]	Making Synthetic Water	Lab gloves, Lab glasses	Sigma-Aldrich	A4514-100G
Calcium Chloride [$CaCl_2$] [s]	Making Synthetic Water	Lab gloves, Lab glasses	Sigma-Aldrich	C4901-500G
Sulfamethoxazole [$C_{10}H_{11}N_3O_3S$] [s]	Antibiotic Feed	Lab gloves, Lab coat, Lab glasses, Care to avoid inhaling, Care to avoid contact with skin	Sigm-Aldrich	S7507-10G
Erythromycin [$C_{37}H_{67}NO_{13}$] [s]	Antibiotic Feed	Lab gloves, Lab coat, Lab glasses, Care to avoid inhaling, Care to avoid contact with skin	Fluka Chemicals	45673
Ciprofloxacin [C17H18FN3O3] [s]	Antibiotic Feed	Lab gloves, Lab coat, Lab glasses, Care to avoid inhaling, Care to avoid contact with skin	Fluka Chemicals	17850
Acetonitrile [CH_3CN or ACN] [l]	Solvent in Mobile Phases	Lab gloves, Lab glasses, Care to avoid inhaling	Sigma-Aldrich	00687-1L
Formic Acid [CH_2O_2] [l]	Solvent in Mobile Phases	Lab gloves, Lab coat, Lab glasses, Care to avoid inhaling, Care to avoid contact with skin	Sigma-Aldrich	F0507-100ML
Deionized water [H_2O] [l]	SPE Process	Lab gloves, Lab glasses	Calvin	SB 050
Methanol [CH_3OH or MeOH] [l]	SPE Process	Lab gloves, Lab coat, Lab glasses, Care to avoid inhaling, Care to avoid contact with skin	Sigma-Aldrich	M3641-1L
Nitrogen [N_2] [g]	Drying Eluates	Lab glasses	Purity Cylinder Gases, Inc. [Bob De Kraker]	UN 1066

Appendix F. Sorption plots and raw data from objective 2 experiments.

Table F.1. Antibiotic Data— CIP (0.33 µg/L, alone)

CIP	
feed cond	0.33 alone
date	19-Jul
k (hr^{-1})	0.1
R^2	0.952
b (Co model)	0.21

raw data			model data		
t	C	C/Co	t	C	C/Co
0	0.00	0.00	0	0.00	0.00
0	0.00	0.00	1	0.02	0.10
1	0.00	0.00	2	0.04	0.18
1	0.00	0.00	3	0.05	0.26
1.5	0.00	0.00	4	0.07	0.33
1.5	0.00	0.00	5	0.08	0.39
2	0.02	0.11	6	0.09	0.45
2	0.01	0.05	7	0.11	0.50
2.5	0.04	0.17	8	0.12	0.55
2.5	0.02	0.11	9	0.12	0.59
3	0.08	0.38	10	0.13	0.63
3	0.03	0.15	11	0.14	0.67
4	0.09	0.41	12	0.15	0.70
4	0.06	0.27	13	0.15	0.73
5	0.12	0.57	14	0.16	0.75
5	0.09	0.42	15	0.16	0.78
7	0.12	0.55	16	0.17	0.80
7	0.11	0.53	17	0.17	0.82
24	0.19	0.92	18	0.18	0.83
24	0.18	0.88	19	0.18	0.85
24.5	0.21	0.98	20	0.18	0.86
24.5	0.19	0.90	21	0.18	0.88
25	0.18	0.88	22	0.19	0.89
25	0.19	0.91	23	0.19	0.90
25.5	0.20	0.95	24	0.19	0.91
25.5	0.19	0.93	25	0.19	0.92

Figure F.1. Effluent Antibiotic Concentration (C/C_0) versus time. CIP (0.33 µg/L, alone)

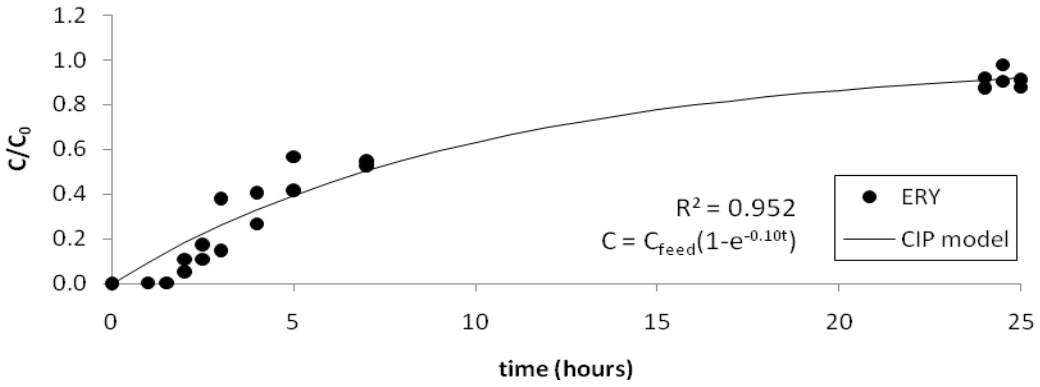


Table F.2. Antibiotic Data— CIP (3.33 µg/L, alone)

CIP	
feed cond	3.33 alone
date	4-Oct
k (hr^{-1})	0.11
R^2	0.815
b (Co model)	2.33

raw data			model data		
t	C	C/Co	t	C	C/Co
0	0.00	0.00	0	0.00	0.00
0	0.00	0.00	1	0.22	0.10
0.5	0.62	0.27	2	0.42	0.18
0.5	0.66	0.28	3	0.60	0.26
1	0.73	0.31	4	0.77	0.33
1	0.62	0.26	5	0.92	0.39
1.5	0.72	0.31	6	1.05	0.45
1.5	0.71	0.31	7	1.17	0.50
2	0.89	0.38	8	1.28	0.55
2	0.75	0.32	9	1.38	0.59
2.5	1.41	0.61	10	1.47	0.63
2.5	1.42	0.61	11	1.55	0.67
4	1.15	0.50	12	1.63	0.70
4	1.16	0.50	13	1.70	0.73
5	1.06	0.45	14	1.76	0.75
5	1.08	0.46	15	1.81	0.78
5.5	1.06	0.46	16	1.86	0.80
5.5	1.17	0.50	17	1.90	0.82
8.5	1.33	0.57	18	1.94	0.83
8.5	1.38	0.59	19	1.98	0.85
24.25	2.27	0.97	20	2.01	0.86
24.25	2.25	0.97	21	2.04	0.88
			22	2.07	0.89
			23	2.10	0.90
			24	2.12	0.91
			25	2.14	0.92

Figure F.2. Effluent Antibiotic Concentration (C/C_0) versus time. CIP (3.33 µg/L, alone)

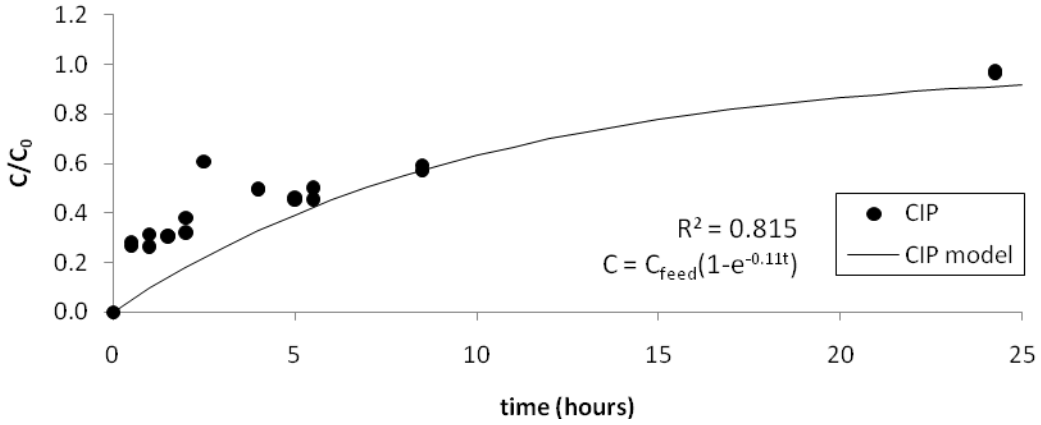


Table F.3. Antibiotic Data— CIP (0.33 µg/L, mixture)

CIP	
feed cond	0.33 mixture
date	26-Jul
k (hr^{-1})	0.11
R^2	0.802
b (Co model)	0.283

raw data			model data		
t	C	C/Co	t	C	C/Co
0	0.00	0.00	0	0.00	0.00
0	0.00	0.00	1	0.03	0.10
2.5	0.03	0.10	2	0.06	0.20
2.5	0.01	0.03	3	0.08	0.28
3	0.11	0.37	4	0.10	0.36
3	0.02	0.06	5	0.12	0.42
4	0.09	0.30	6	0.14	0.48
4	0.06	0.23	7	0.15	0.54
5	0.12	0.43	8	0.17	0.59
5	0.10	0.36	9	0.18	0.63
7	0.26	0.91	10	0.19	0.67
7	0.16	0.55	11	0.20	0.70
24	0.25	0.90	12	0.21	0.73
24	0.25	0.89	13	0.22	0.76
			14	0.22	0.79
			15	0.23	0.81
			16	0.23	0.83
			17	0.24	0.85
			18	0.24	0.86
			19	0.25	0.88
			20	0.25	0.89
			21	0.25	0.90
			22	0.26	0.91
			23	0.26	0.92
			24	0.26	0.93
			25	0.26	0.94

Figure F.3. Effluent Antibiotic Concentration (C/C_0) versus time. CIP (0.33 µg/L, mixture)

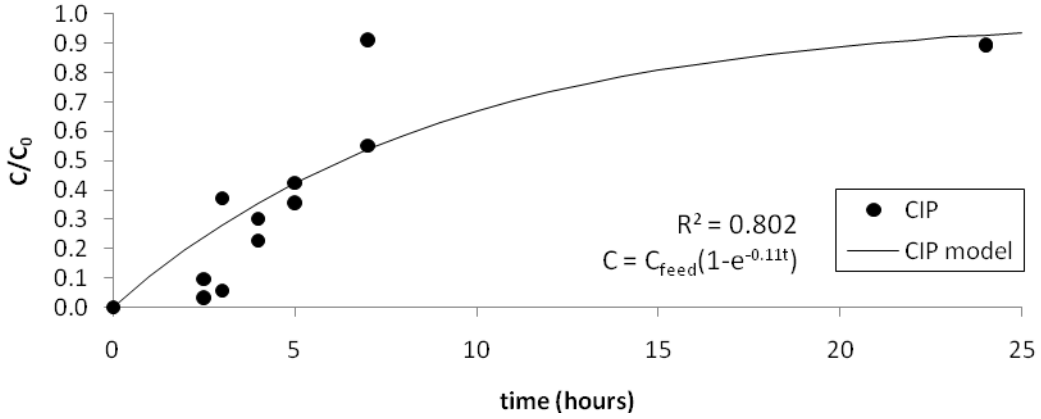


Table F.4. Antibiotic Data— CIP (0.33 µg/L, mixture)

CIP	
feed cond	0.33 mixture
date	29-Aug
k (hr^{-1})	0.09
R^2	0.979
b (Co model)	0.274

raw data			model data		
t	C	C/Co	t	C	C/Co
0	0.00	0.00	0	0.00	0.00
0	0.00	0.00	1	0.02	0.09
1	0.03	0.11	2	0.05	0.16
1	0.03	0.11	3	0.06	0.24
1.5	0.04	0.13	4	0.08	0.30
1.5	0.04	0.13	5	0.10	0.36
2	0.05	0.17	6	0.11	0.42
2	0.03	0.10	7	0.13	0.47
2.5	0.08	0.28	8	0.14	0.51
2.5	0.06	0.21	9	0.15	0.56
3.5	0.09	0.33	10	0.16	0.59
3.5	0.06	0.23	11	0.17	0.63
4.5	0.09	0.32	12	0.18	0.66
4.5	0.08	0.30	13	0.19	0.69
6.5	0.11	0.40	14	0.20	0.72
6.5	0.11	0.40	15	0.20	0.74
8.5	0.17	0.61	16	0.21	0.76
8.5	0.15	0.55	17	0.21	0.78
10.5	0.19	0.70	18	0.22	0.80
10.5	0.17	0.61	19	0.22	0.82
12.5	0.19	0.70	20	0.23	0.83
12.5	0.18	0.66	21	0.23	0.85
14.5	0.21	0.77	22	0.24	0.86
14.5	0.20	0.73	23	0.24	0.87
21.5	0.24	0.89	24	0.24	0.88
21.5	0.26	0.95	25	0.25	0.89
24.5	0.22	0.82			
24.5	0.24	0.87			

Figure F.4. Effluent Antibiotic Concentration (C/C_0) versus time. CIP (0.33 µg/L, mixture)

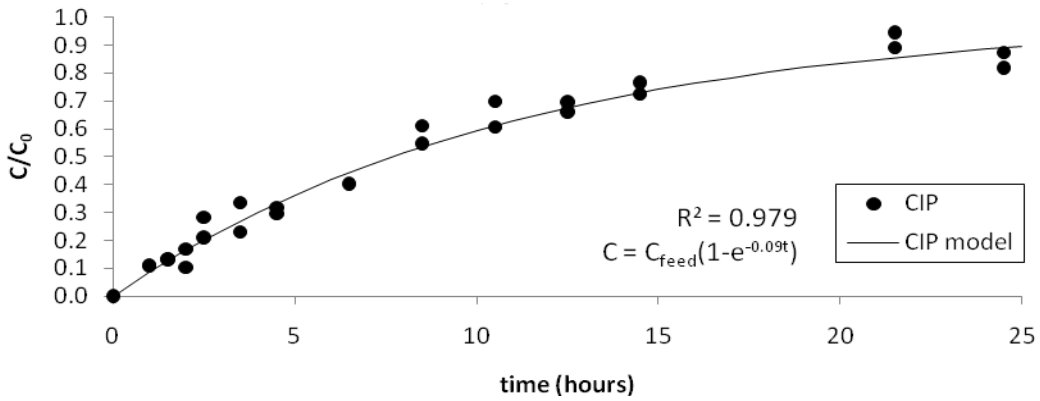


Table F.5. Antibiotic Data— CIP (3.33 µg/L, mixture)

CIP	
feed cond	3.33 mixture
date	8-Nov
k (hr^{-1})	0.06
R^2	0.976
b (Co model)	2.81

raw data			model data		
t	C	C/Co	t	C	C/Co
0	0.00	0.00	0	0.00	0.00
0	0.00	0.00	1	0.16	0.06
1	0.47	0.17	2	0.32	0.11
1	0.43	0.15	3	0.46	0.16
1.5	0.51	0.18	4	0.60	0.21
1.5	0.49	0.17	5	0.73	0.26
2	0.59	0.21	6	0.85	0.30
2	0.58	0.21	7	0.96	0.34
2.5	0.80	0.28	8	1.07	0.38
2.5	0.81	0.29	9	1.17	0.42
3	0.72	0.26	10	1.27	0.45
3	0.88	0.31	11	1.36	0.48
3.5	0.73	0.26	12	1.44	0.51
3.5	0.80	0.29	13	1.52	0.54
5	1.02	0.36	14	1.60	0.57
5	1.11	0.40	15	1.67	0.59
6	0.89	0.32	16	1.73	0.62
6	0.96	0.34	17	1.80	0.64
7	1.22	0.43	18	1.86	0.66
7	1.18	0.42	19	1.91	0.68
9.5	1.36	0.48	20	1.96	0.70
9.5	1.35	0.48	21	2.01	0.72
14	1.67	0.59	22	2.06	0.73
14	1.64	0.58	23	2.10	0.75
24.5	2.15	0.76	24	2.14	0.76
24.5	2.14	0.76	25	2.18	0.78

Figure F.5. Effluent Antibiotic Concentration (C/C_0) versus time. CIP (3.33 µg/L, mixture)

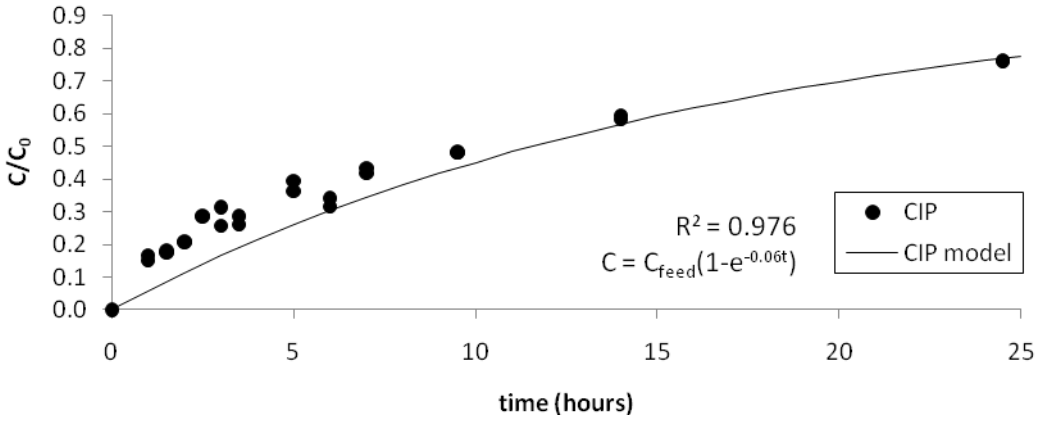


Table F.6. Antibiotic Data— CIP (3.33 µg/L, mixture)

CIP	
feed cond	3.33 mixture
date	11-Oct
k (hr^{-1})	0.06
R^2	0.992
b (Co model)	2.73

raw data			model data		
t	C	C/Co	t	C	C/Co
0	0.00	0.00	0	0.00	0.00
0	0.00	0.00	1	0.16	0.06
1	0.31	0.11	2	0.31	0.11
1	0.21	0.08	3	0.45	0.16
1.5	0.22	0.08	4	0.58	0.21
1.5	0.22	0.08	5	0.71	0.26
2	0.36	0.13	6	0.83	0.30
2	0.36	0.13	7	0.94	0.34
2.5	0.56	0.21	8	1.04	0.38
2.5	0.40	0.15	9	1.14	0.42
3	0.54	0.20	10	1.23	0.45
3	0.49	0.18	11	1.32	0.48
3.5	0.57	0.21	12	1.40	0.51
3.5	0.56	0.20	13	1.48	0.54
5	0.86	0.31	14	1.55	0.57
5	0.78	0.29	15	1.62	0.59
6	0.92	0.34	16	1.68	0.62
6	0.88	0.32	17	1.75	0.64
9	1.21	0.44	18	1.80	0.66
9	1.14	0.42	19	1.86	0.68
24.5	2.19	0.80	20	1.91	0.70
24.5	2.17	0.80	21	1.96	0.72
			22	2.00	0.73
			23	2.04	0.75
			24	2.08	0.76
			25	2.12	0.78

Figure F.6. Effluent Antibiotic Concentration (C/C_0) versus time. CIP (3.33 µg/L, mixture)

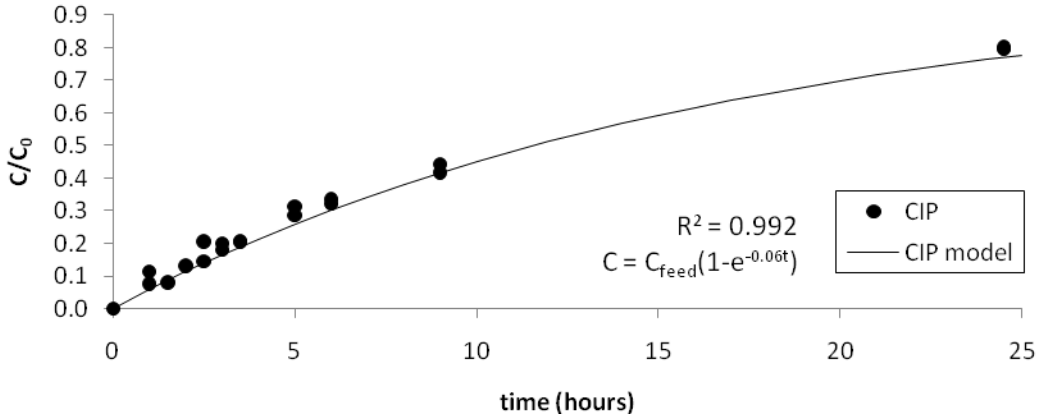


Table F.7. Antibiotic Data— ERY (0.33 µg/L, alone)

ERY	
feed cond	0.33 alone
date	13-Jul
k (hr^{-1})	0.95
R^2	0.706
b (Co model)	0.355

raw data			model data		
t	C	C/Co	t	C	C/Co
0	0.00	0.00	0	0.00	0.00
0	0.00	0.00	1	0.22	0.61
1	0.11	0.32	2	0.30	0.85
1	0.11	0.31	3	0.33	0.94
1.5	0.25	0.70	4	0.35	0.98
1.5	0.25	0.70	5	0.35	0.99
2.5	0.43	1.21	6	0.35	1.00
2.5	0.39	1.09	7	0.35	1.00
3	0.48	1.35	8	0.35	1.00
3	0.48	1.35	9	0.35	1.00
5	0.35	1.00	10	0.35	1.00
5	0.31	0.87	11	0.35	1.00
7	0.30	0.85	12	0.35	1.00
7	0.31	0.87	13	0.35	1.00
24	0.27	0.77	14	0.35	1.00
24.5	0.36	1.02	15	0.35	1.00
25	0.28	0.78	16	0.35	1.00
			17	0.35	1.00
			18	0.35	1.00
			19	0.35	1.00
			20	0.35	1.00
			21	0.35	1.00
			22	0.35	1.00
			23	0.35	1.00
			24	0.35	1.00
			25	0.35	1.00

Figure F.7. Effluent Antibiotic Concentration (C/C_0) versus time. ERY (0.33 µg/L, alone)

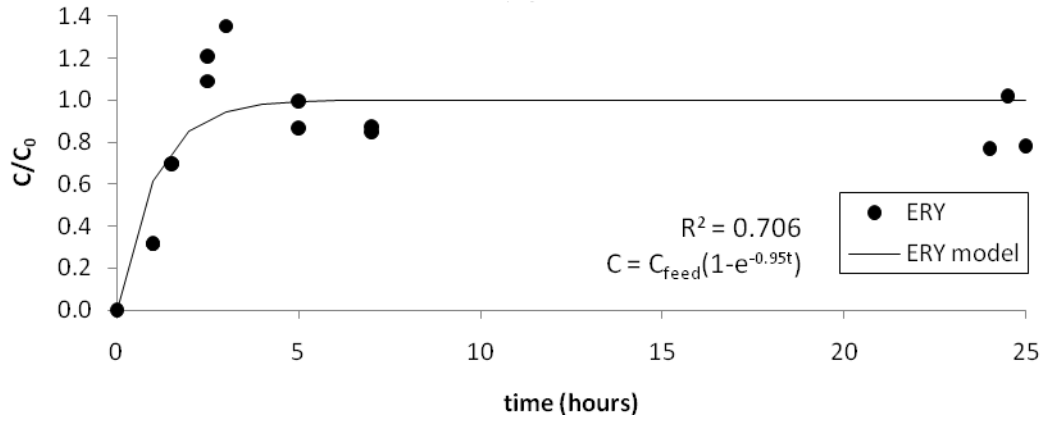


Table F.8. Antibiotic Data— ERY (3.33 µg/L, alone)

ERY	
feed cond	3.33 alone
date	20-Sep
k (hr^{-1})	0.74
R^2	0.925
b (Co model)	3.19

raw data			model data		
t	C	C/Co	t	C	C/Co
0	0.00	0.00	0	0.00	0.00
0	0.00	0.00	1	1.67	0.52
1	1.47	0.46	2	2.46	0.77
1	1.46	0.46	3	2.84	0.89
1.5	2.27	0.71	4	3.02	0.95
1.5	2.28	0.71	5	3.11	0.98
2	2.56	0.80	6	3.15	0.99
2	2.55	0.80	7	3.17	0.99
2.5	3.10	0.97	8	3.18	1.00
2.5	2.01	0.63	9	3.19	1.00
3	2.87	0.90	10	3.19	1.00
3	2.91	0.91	11	3.19	1.00
5	3.23	1.01	12	3.19	1.00
5	2.79	0.88	13	3.19	1.00
6	3.36	1.05	14	3.19	1.00
6	3.35	1.05	15	3.19	1.00
8.5	3.31	1.04	16	3.19	1.00
8.5	3.32	1.04	17	3.19	1.00
24.5	2.73	0.86	18	3.19	1.00
24.5	2.74	0.86	19	3.19	1.00
25	3.58	1.12	20	3.19	1.00
25	3.18	1.00	21	3.19	1.00
			22	3.19	1.00
			23	3.19	1.00
			24	3.19	1.00
			25	3.19	1.00

Figure F.8. Effluent Antibiotic Concentration (C/C_0) versus time. ERY (3.33 µg/L, alone)

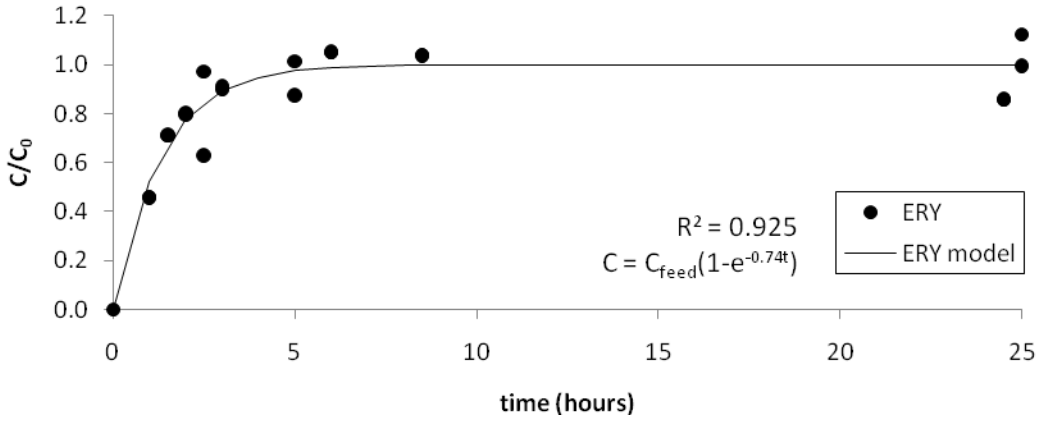


Table F.9. Antibiotic Data— ERY (0.33 µg/L, mixture)

ERY	
	0.33
feed cond	mixture
date	26-Jul
k (hr^{-1})	0.69
R^2	0.703
b (Co model)	0.312

raw data			model data		
t	C	C/Co	t	C	C/Co
0	0.00	0.00	0	0.00	0.00
0	0.00	0.00	1	0.16	0.50
1	0.05	0.16	2	0.23	0.75
1	0.05	0.15	3	0.27	0.87
1.5	0.18	0.57	4	0.29	0.94
1.5	0.17	0.54	5	0.30	0.97
2	0.22	0.69	6	0.31	0.98
2	0.21	0.67	7	0.31	0.99
2.5	0.36	1.15	8	0.31	1.00
2.5	0.37	1.18	9	0.31	1.00
3	0.34	1.08	10	0.31	1.00
3	0.34	1.08	11	0.31	1.00
4	0.28	0.91	12	0.31	1.00
4	0.27	0.87	13	0.31	1.00
5	0.38	1.21	14	0.31	1.00
5	0.39	1.23	15	0.31	1.00
7	0.27	0.87	16	0.31	1.00
7	0.25	0.81	17	0.31	1.00
24	0.23	0.75	18	0.31	1.00
24	0.24	0.77	19	0.31	1.00
			20	0.31	1.00
			21	0.31	1.00
			22	0.31	1.00
			23	0.31	1.00
			24	0.31	1.00
			25	0.31	1.00

Figure F.9. Effluent Antibiotic Concentration (C/C_0) versus time. ERY (0.33 µg/L, mixture)

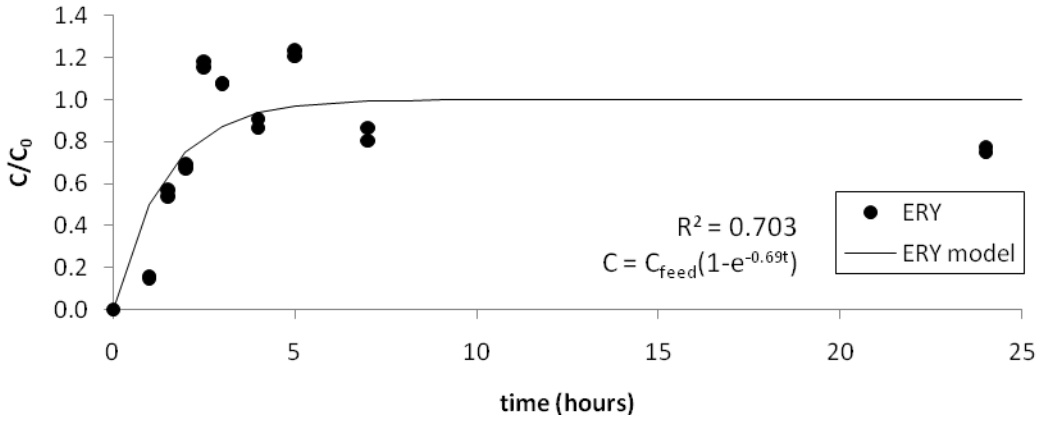


Table F.10. Antibiotic Data— ERY (0.33 µg/L, mixture)

ERY	
	0.33
feed cond	mixture
date	29-Aug
k (hr^{-1})	0.66
R^2	0.969
b (Co model)	0.352

raw data			model data		
t	C	C/Co	t	C	C/Co
0	0.00	0.00	0	0.00	0.00
0	0.00	0.00	1	0.17	0.48
1	0.12	0.35	2	0.26	0.73
1	0.12	0.33	3	0.30	0.86
1.5	0.21	0.61	4	0.33	0.93
1.5	0.21	0.60	5	0.34	0.96
2	0.29	0.81	6	0.35	0.98
2	0.28	0.79	7	0.35	0.99
2.5	0.30	0.87	8	0.35	0.99
2.5	0.30	0.85	9	0.35	1.00
3.5	0.33	0.93	10	0.35	1.00
3.5	0.32	0.91	11	0.35	1.00
4.5	0.33	0.94	12	0.35	1.00
4.5	0.34	0.97	13	0.35	1.00
6.5	0.35	1.00	14	0.35	1.00
6.5	0.35	1.00	15	0.35	1.00
8.5	0.36	1.01	16	0.35	1.00
8.5	0.36	1.02	17	0.35	1.00
10.5	0.35	1.01	18	0.35	1.00
10.5	0.35	1.01	19	0.35	1.00
12.5	0.34	0.95	20	0.35	1.00
12.5	0.35	1.00	21	0.35	1.00
14.5	0.37	1.04	22	0.35	1.00
14.5	0.35	1.00	23	0.35	1.00
21.5	0.33	0.94	24	0.35	1.00
21.5	0.35	1.00	25	0.35	1.00
24.5	0.32	0.92			
24.5	0.34	0.96			

Figure F.10. Effluent Antibiotic Concentration (C/C_0) versus time. ERY (0.33 µg/L, mixture)

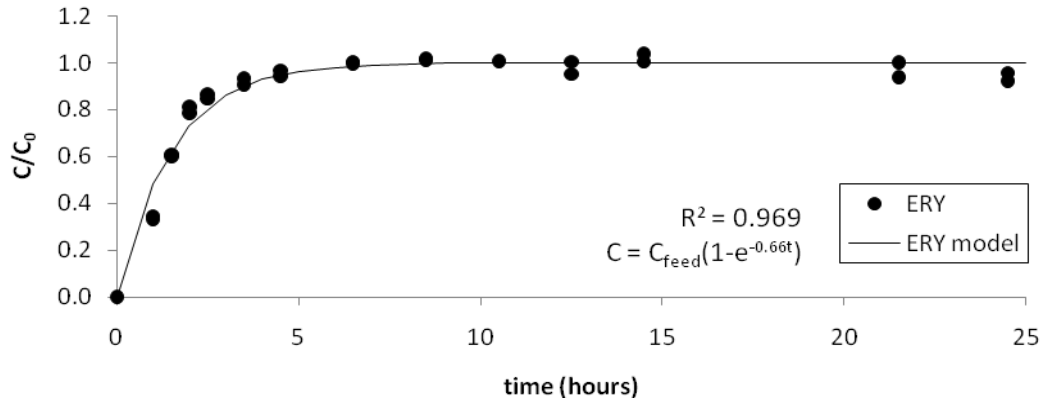


Table F.11. Antibiotic Data— ERY (3.33 µg/L, mixture)

ERY	
feed cond	3.33mixture
date	1-Nov
k (hr^{-1})	1.25
R^2	0.601
b (Co model)	6.09

raw data			model data		
t	C	C/Co	t	C	C/Co
1	4.29	0.70	0	0.00	0.00
1	4.05	0.67	1	4.35	0.71
1.5	5.22	0.86	2	5.59	0.92
1.5	5.13	0.84	3	5.95	0.98
2.5	5.77	0.95	4	6.05	0.99
2.5	6.03	0.99	5	6.08	1.00
3.5	5.80	0.95	6	6.09	1.00
3.5	6.47	1.06	7	6.09	1.00
4.5	6.02	0.99	8	6.09	1.00
4.5	6.83	1.12	9	6.09	1.00
5.5	5.76	0.95	10	6.09	1.00
5.5	6.60	1.08	11	6.09	1.00
6.5	5.42	0.89	12	6.09	1.00
6.5	5.43	0.89	13	6.09	1.00
7.5	6.25	1.03	14	6.09	1.00
7.5	6.11	1.00	15	6.09	1.00
8.5	6.98	1.15	16	6.09	1.00
8.5	6.72	1.10	17	6.09	1.00
12.5	5.42	0.89	18	6.09	1.00
12.5	5.37	0.88	19	6.09	1.00
24.5	6.33	1.04	20	6.09	1.00
24.5	5.57	0.91	21	6.09	1.00
			22	6.09	1.00
			23	6.09	1.00
			24	6.09	1.00
			25	6.09	1.00

Figure F.11. Effluent Antibiotic Concentration (C/C_0) versus time. ERY (3.33 µg/L, mixture)

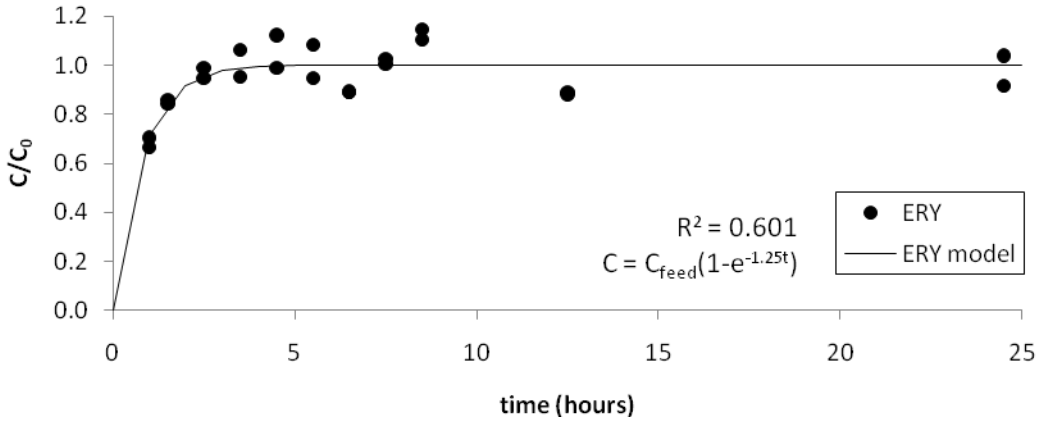


Table F.12. Antibiotic Data— ERY (3.33 µg/L, mixture)

ERY	
	3.33
feed cond	mixture
date	11-Oct
k (hr^{-1})	0.68
R^2	0.836
b (Co model)	4.3

raw data			model data		
t	C	C/Co	t	C	C/Co
0	0.00	0.00	0	0.00	0.00
0	0.00	0.00	1	2.12	0.49
1	1.98	0.46	2	3.20	0.74
1	1.97	0.46	3	3.74	0.87
1.5	3.48	0.81	4	4.02	0.93
1.5	2.86	0.66	5	4.16	0.97
2	3.00	0.70	6	4.23	0.98
2	2.98	0.69	7	4.26	0.99
2.5	3.97	0.92	8	4.28	1.00
2.5	4.25	0.99	9	4.29	1.00
3	4.26	0.99	10	4.30	1.00
3	3.83	0.89	11	4.30	1.00
3.5	3.17	0.74	12	4.30	1.00
3.5	3.28	0.76	13	4.30	1.00
5	3.06	0.71	14	4.30	1.00
5	3.12	0.73	15	4.30	1.00
6	4.37	1.02	16	4.30	1.00
6	4.42	1.03	17	4.30	1.00
9	4.09	0.95	18	4.30	1.00
9	4.35	1.01	19	4.30	1.00
24.5	5.24	1.22	20	4.30	1.00
24.5	5.08	1.18	21	4.30	1.00
			22	4.30	1.00
			23	4.30	1.00
			24	4.30	1.00
			25	4.30	1.00

Figure F.12. Effluent Antibiotic Concentration (C/C_0) versus time. ERY (3.33 µg/L, mixture)

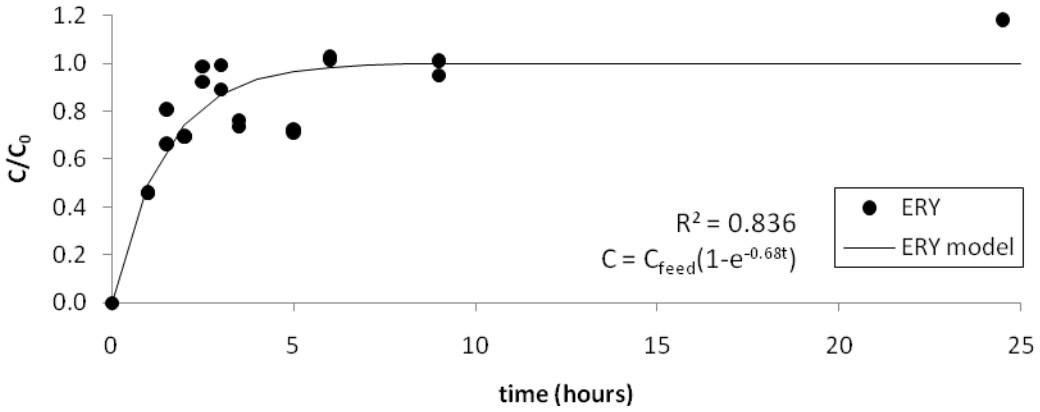


Table F.13. Antibiotic Data— SMX (0.33 µg/L, alone)

SMX	
feed cond	0.33 alone
date	5-Jul
k (hr^{-1})	0.72
R^2	0.946
b (Co model)	0.172

raw data			model data		
t	C	C/Co	t	C	C/Co
0	0.00	0.00	0	0.00	0.00
0	0.00	0.00	1	0.09	0.51
1	0.06	0.33	2	0.13	0.76
1	0.06	0.33	3	0.15	0.88
2	0.15	0.85	4	0.16	0.94
2	0.14	0.83	5	0.17	0.97
2.5	0.16	0.91	6	0.17	0.99
2.5	0.16	0.92	7	0.17	0.99
3	0.16	0.91	8	0.17	1.00
3	0.16	0.90	9	0.17	1.00
4	0.16	0.95	10	0.17	1.00
4	0.17	0.97	11	0.17	1.00
7	0.17	1.02	12	0.17	1.00
7	0.17	1.02	13	0.17	1.00
24	0.16	0.93	14	0.17	1.00
24	0.16	0.92	15	0.17	1.00
24.5	0.17	0.97	16	0.17	1.00
24.5	0.17	0.98	17	0.17	1.00
			18	0.17	1.00
			19	0.17	1.00
			20	0.17	1.00
			21	0.17	1.00
			22	0.17	1.00
			23	0.17	1.00
			24	0.17	1.00
			25	0.17	1.00

Figure F.13. Effluent Antibiotic Concentration (C/C_0) versus time. SMX (0.33 µg/L, alone)

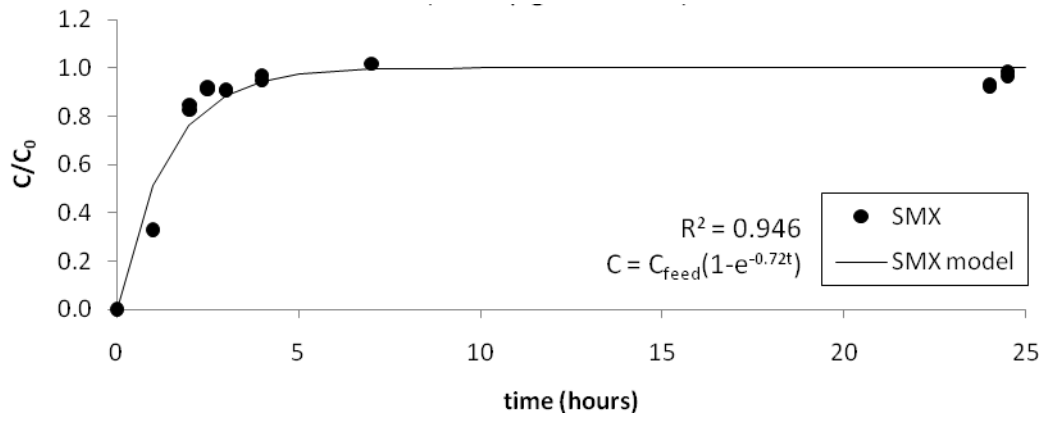


Table F.14. Antibiotic Data— SMX (3.33 µg/L, alone)

SMX	
feed cond	3.33 alone
date	13-Sep
k (hr^{-1})	3.49
R^2	0.883
b (Co model)	3.56

raw data			model data		
t	C	C/Co	t	C	C/Co
0	0.00	0.00	0	0.00	0.00
0	0.00	0.00	1	3.45	0.97
0.5	2.87	0.80	2	3.56	1.00
0.5	2.97	0.83	3	3.56	1.00
1	3.51	0.99	4	3.56	1.00
1	3.45	0.97	5	3.56	1.00
1.5	3.50	0.98	6	3.56	1.00
1.5	3.54	0.99	7	3.56	1.00
2	3.84	1.08	8	3.56	1.00
2	3.93	1.10	9	3.56	1.00
2.5	3.88	1.09	10	3.56	1.00
2.5	3.90	1.10	11	3.56	1.00
3	3.73	1.05	12	3.56	1.00
3	3.68	1.04	13	3.56	1.00
5	3.78	1.06	14	3.56	1.00
5	3.60	1.01	15	3.56	1.00
6	2.42	0.68	16	3.56	1.00
6	2.55	0.72	17	3.56	1.00
9	3.41	0.96	18	3.56	1.00
9	3.43	0.96	19	3.56	1.00
24.5	3.79	1.06	20	3.56	1.00
24.5	3.83	1.08	21	3.56	1.00
			22	3.56	1.00
			23	3.56	1.00
			24	3.56	1.00
			25	3.56	1.00

Figure F.14. Effluent Antibiotic Concentration (C/C_0) versus time. SMX (3.33 µg/L, alone)

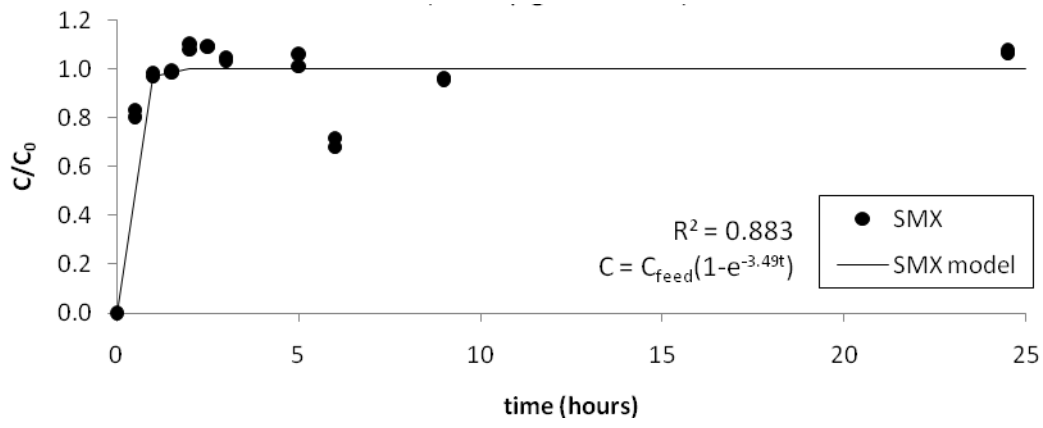


Table F.15. Antibiotic Data— SMX (0.33 µg/L, mixture)

SMX	
feed cond	0.33 mixture
date	26-Jul
k (hr^{-1})	0.97
R^2	0.414
b (Co model)	0.256

raw data			model data		
t	C	C/Co	t	C	C/Co
0	0.00	0.00	0	0.00	0.00
0	0.00	0.00	1	0.16	0.62
1	0.05	0.21	2	0.22	0.86
1	0.05	0.20	3	0.24	0.95
1.5	0.22	0.86	4	0.25	0.98
1.5	0.22	0.86	5	0.25	0.99
2	0.14	0.56	6	0.26	1.00
2	0.14	0.56	7	0.26	1.00
2.5	0.45	1.76	8	0.26	1.00
2.5	0.45	1.75	9	0.26	1.00
3	0.31	1.19	10	0.26	1.00
3	0.30	1.15	11	0.26	1.00
4	0.18	0.69	12	0.26	1.00
4	0.17	0.67	13	0.26	1.00
5	0.34	1.34	14	0.26	1.00
5	0.35	1.35	15	0.26	1.00
7	0.18	0.69	16	0.26	1.00
7	0.18	0.69	17	0.26	1.00
24	0.18	0.71	18	0.26	1.00
24	0.18	0.70	19	0.26	1.00
			20	0.26	1.00
			21	0.26	1.00
			22	0.26	1.00
			23	0.26	1.00
			24	0.26	1.00
			25	0.26	1.00

Figure F.15. Effluent Antibiotic Concentration (C/C_0) versus time. SMX (0.33 µg/L, mixture)

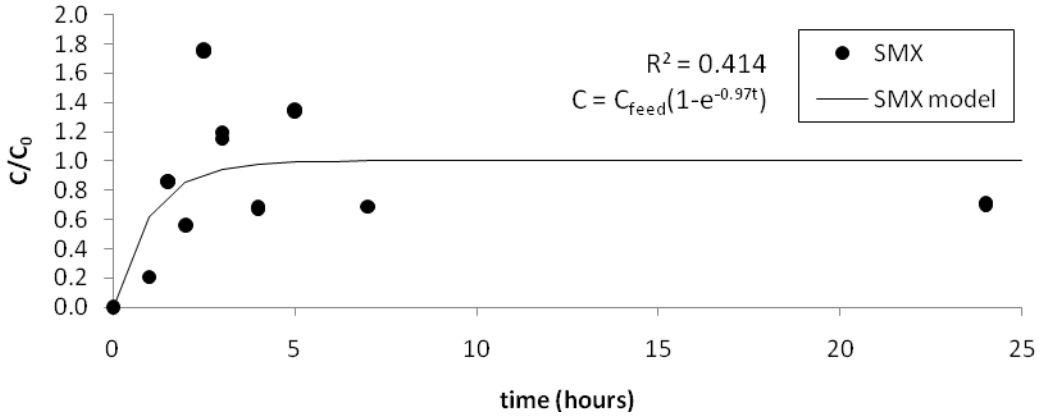


Table F.16. Antibiotic Data— SMX (0.33 µg/L, mixture)

SMX	
feed cond	0.33 mixture
date	29-Aug
k (hr^{-1})	0.74
R^2	0.941
b (Co model)	0.324

raw data			model data		
t	C	C/Co	t	C	C/Co
0	0.00	0.00	0	0.00	0.00
0	0.00	0.00	1	0.17	0.52
1	0.10	0.31	2	0.25	0.77
1	0.10	0.31	3	0.29	0.89
1.5	0.22	0.67	4	0.31	0.95
1.5	0.22	0.67	5	0.32	0.98
2	0.27	0.85	6	0.32	0.99
2	0.27	0.84	7	0.32	0.99
2.5	0.30	0.91	8	0.32	1.00
2.5	0.29	0.90	9	0.32	1.00
3.5	0.32	0.99	10	0.32	1.00
3.5	0.31	0.97	11	0.32	1.00
4.5	0.32	0.97	12	0.32	1.00
4.5	0.32	0.98	13	0.32	1.00
6.5	0.34	1.04	14	0.32	1.00
6.5	0.33	1.03	15	0.32	1.00
8.5	0.34	1.05	16	0.32	1.00
8.5	0.33	1.03	17	0.32	1.00
10.5	0.31	0.97	18	0.32	1.00
10.5	0.32	0.97	19	0.32	1.00
12.5	0.31	0.95	20	0.32	1.00
12.5	0.31	0.94	21	0.32	1.00
14.5	0.30	0.94	22	0.32	1.00
14.5	0.32	0.98	23	0.32	1.00
21.5	0.31	0.96	24	0.32	1.00
21.5	0.31	0.94	25	0.32	1.00
24.5	0.32	0.99			
24.5	0.32	1.00			

Figure F.16. Effluent Antibiotic Concentration (C/C_0) versus time. SMX (0.33 µg/L, mixture)

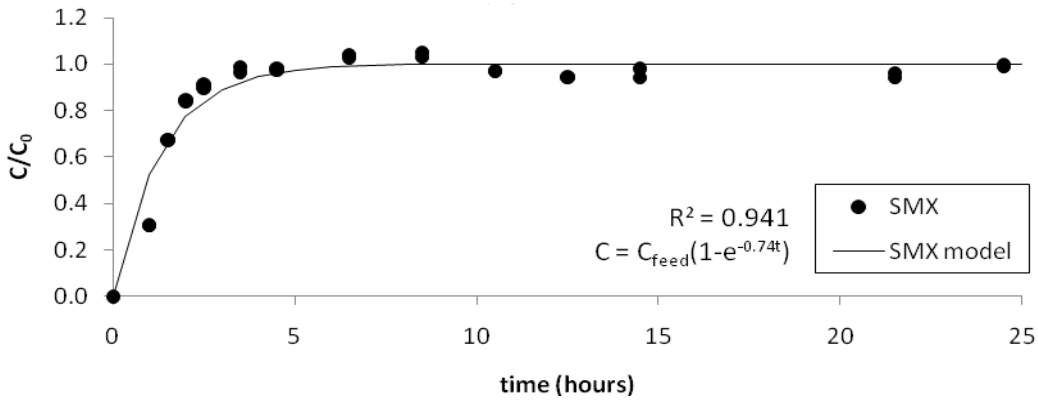


Table F.17. Antibiotic Data— SMX (3.33 µg/L, mixture)

SMX	
feed cond	3.33 mixture
date	11-Oct
k (hr^{-1})	1.05
R^2	0.988
b (Co model)	4.17

raw data			model data		
t	C	C/Co	t	C	C/Co
0	0.00	0.00	0	0.00	0.00
0	0.00	0.00	1	2.71	0.65
1	2.42	0.58	2	3.66	0.88
1	2.37	0.57	3	3.99	0.96
1.5	3.44	0.82	4	4.11	0.99
1.5	3.36	0.81	5	4.15	0.99
2	3.85	0.92	6	4.16	1.00
2	3.82	0.92	7	4.17	1.00
2.5	3.98	0.95	8	4.17	1.00
2.5	3.97	0.95	9	4.17	1.00
3	4.03	0.97	10	4.17	1.00
3	4.06	0.97	11	4.17	1.00
3.5	4.06	0.97	12	4.17	1.00
3.5	4.05	0.97	13	4.17	1.00
5	4.13	0.99	14	4.17	1.00
5	4.10	0.98	15	4.17	1.00
6	4.09	0.98	16	4.17	1.00
6	4.06	0.97	17	4.17	1.00
9	4.03	0.97	18	4.17	1.00
9	4.05	0.97	19	4.17	1.00
24.5	4.20	1.01	20	4.17	1.00
24.5	4.20	1.01	21	4.17	1.00
			22	4.17	1.00
			23	4.17	1.00
			24	4.17	1.00
			25	4.17	1.00

Figure F.17. Effluent Antibiotic Concentration (C/C_0) versus time. SMX (3.33 µg/L, mixture)

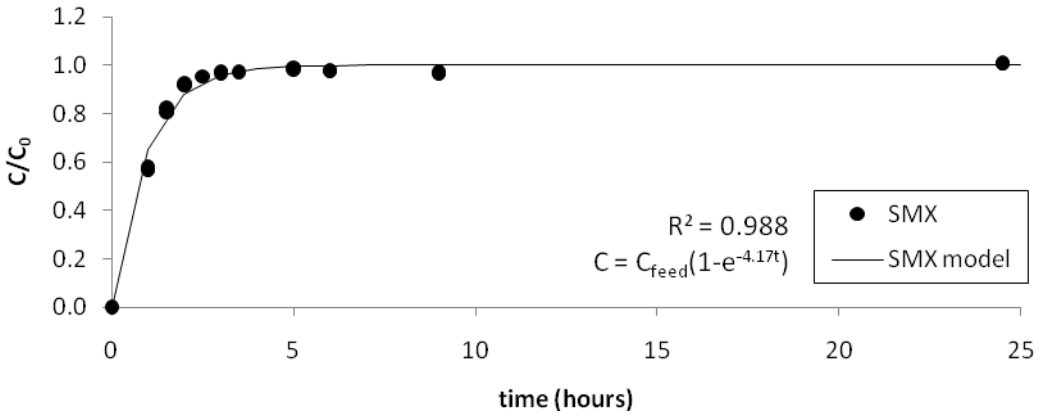


Table F.18. Antibiotic Data— SMX (3.33 µg/L, mixture)

SMX	
	3.33
feed cond	mixture
date	8-Nov
k (hr^{-1})	2.41
R^2	0.954
b (Co model)	3.44

raw data			model data		
t	C	C/Co	t	C	C/Co
0	0.00	0.00	0	0.00	0.00
0	0.00	0.00	1	3.13	0.91
1	3.13	0.91	2	3.41	0.99
1	3.16	0.92	3	3.44	1.00
1.5	3.33	0.97	4	3.44	1.00
1.5	3.33	0.97	5	3.44	1.00
2	3.25	0.94	6	3.44	1.00
2	3.25	0.94	7	3.44	1.00
2.5	3.90	1.13	8	3.44	1.00
2.5	3.92	1.14	9	3.44	1.00
3	3.31	0.96	10	3.44	1.00
3	3.32	0.96	11	3.44	1.00
3.5	3.23	0.94	12	3.44	1.00
3.5	3.22	0.94	13	3.44	1.00
5	3.55	1.03	14	3.44	1.00
5	3.51	1.02	15	3.44	1.00
6	3.13	0.91	16	3.44	1.00
6	3.13	0.91	17	3.44	1.00
7	3.54	1.03	18	3.44	1.00
7	3.52	1.02	19	3.44	1.00
9.5	3.49	1.01	20	3.44	1.00
9.5	3.46	1.01	21	3.44	1.00
14	3.60	1.05	22	3.44	1.00
14	3.62	1.05	23	3.44	1.00
24.5	3.37	0.98	24	3.44	1.00
24.5	3.33	0.97	25	3.44	1.00

Figure F.18. Effluent Antibiotic Concentration (C/C_0) versus time. SMX (3.33 µg/L, mixture)

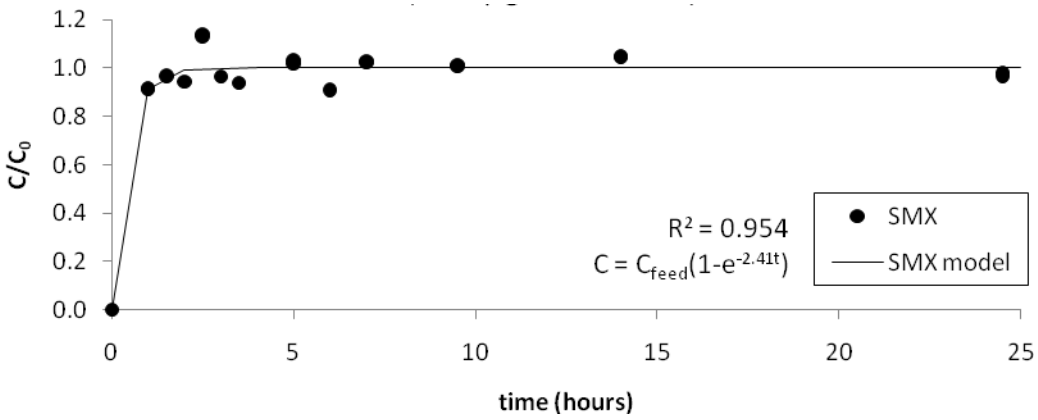


Table F.19. Antibiotic Data— SMX (3.33 µg/L, mixture, w/ 1 mg/L NOM)

SMX	
feed cond	3.33 mixture w/ 1 mg/L NOM
date	29-Nov
k (hr^{-1})	1.28
R^2	0.975
b (Co model)	3.08

raw data			model data		
t	C	C/Co	t	C	C/Co
0	0.00	0.00	0	0.00	0.00
0	0.00	0.00	1	2.22	0.72
1	2.41	0.78	2	2.84	0.92
1	2.38	0.77	3	3.01	0.98
1.5	2.68	0.87	4	3.06	0.99
1.5	2.70	0.88	5	3.07	1.00
2	2.58	0.84	6	3.08	1.00
2	2.62	0.85	7	3.08	1.00
2.5	2.86	0.93	8	3.08	1.00
2.5	2.82	0.92	9	3.08	1.00
3	2.99	0.97	10	3.08	1.00
3	3.03	0.98	11	3.08	1.00
4	2.88	0.94	12	3.08	1.00
4	2.88	0.93	13	3.08	1.00
5	2.91	0.95	14	3.08	1.00
5	2.95	0.96	15	3.08	1.00
6	3.09	1.00	16	3.08	1.00
6	3.11	1.01	17	3.08	1.00
7	3.20	1.04	18	3.08	1.00
7	3.14	1.02	19	3.08	1.00
9	3.14	1.02	20	3.08	1.00
9	3.16	1.03	21	3.08	1.00
12.5	3.05	0.99	22	3.08	1.00
12.5	3.03	0.99	23	3.08	1.00
25	3.14	1.02	24	3.08	1.00
25	3.14	1.02	25	3.08	1.00
26	3.26	1.06			
26	3.27	1.06			
27	3.15	1.02			
27	3.22	1.05			

Figure F.19. Effluent Antibiotic Concentration (C/C_0) versus time. SMX (3.33 µg/L, mixture, w/ 1 mg/L NOM)

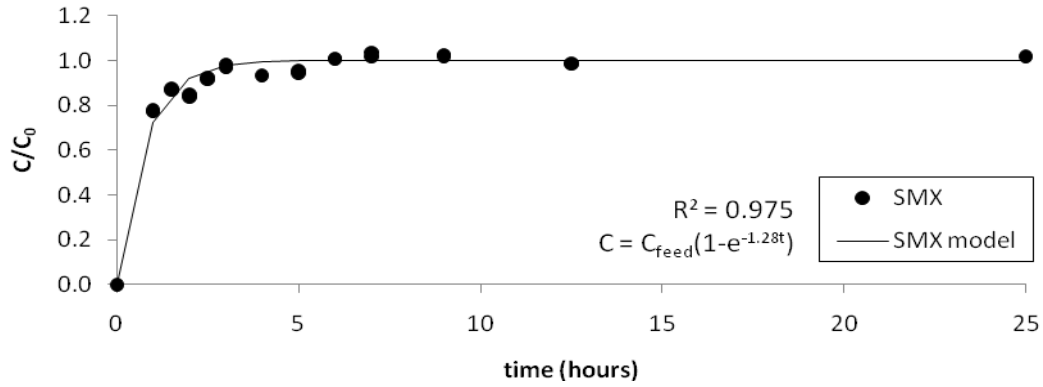


Table F.20. Antibiotic Data— ERY (3.33 µg/L, mixture, w/ 1 mg/L NOM)

ERY	
feed cond	3.33 mixture w/ 1 mg/L NOM
date	29-Nov
k (hr^{-1})	1.55
R^2	0.696
b (Co model)	2.45

raw data			model data		
t	C	C/Co	t	C	C/Co
0	0.00	0.00	0	0.00	0.00
0	0.00	0.00	1	1.93	0.79
1	2.42	0.99	2	2.34	0.95
1	1.11	0.45	3	2.43	0.99
1.5	2.25	0.92	4	2.45	1.00
1.5	2.25	0.92	5	2.45	1.00
2	2.45	1.00	6	2.45	1.00
2	2.39	0.98	7	2.45	1.00
2.5	2.73	1.11	8	2.45	1.00
2.5	2.44	1.00	9	2.45	1.00
3	2.70	1.10	10	2.45	1.00
3	2.71	1.10	11	2.45	1.00
4	2.76	1.13	12	2.45	1.00
4	2.74	1.12	13	2.45	1.00
5	1.59	0.65	14	2.45	1.00
5	2.29	0.94	15	2.45	1.00
6	2.56	1.05	16	2.45	1.00
6	2.70	1.10	17	2.45	1.00
7	3.11	1.27	18	2.45	1.00
7	3.08	1.26	19	2.45	1.00
9	2.45	1.00	20	2.45	1.00
9	3.05	1.25	21	2.45	1.00
12.5	1.98	0.81	22	2.45	1.00
12.5	2.62	1.07	23	2.45	1.00
25	2.34	0.95	24	2.45	1.00
25	1.93	0.79	25	2.45	1.00

Figure F.20. Effluent Antibiotic Concentration (C/C_0) versus time. CIP (3.33 µg/L, mixture, w/ 1 mg/L NOM)

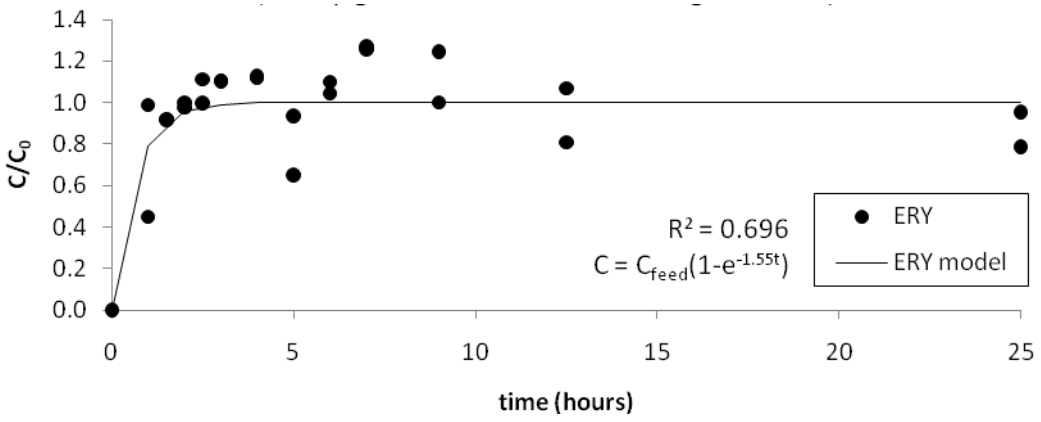
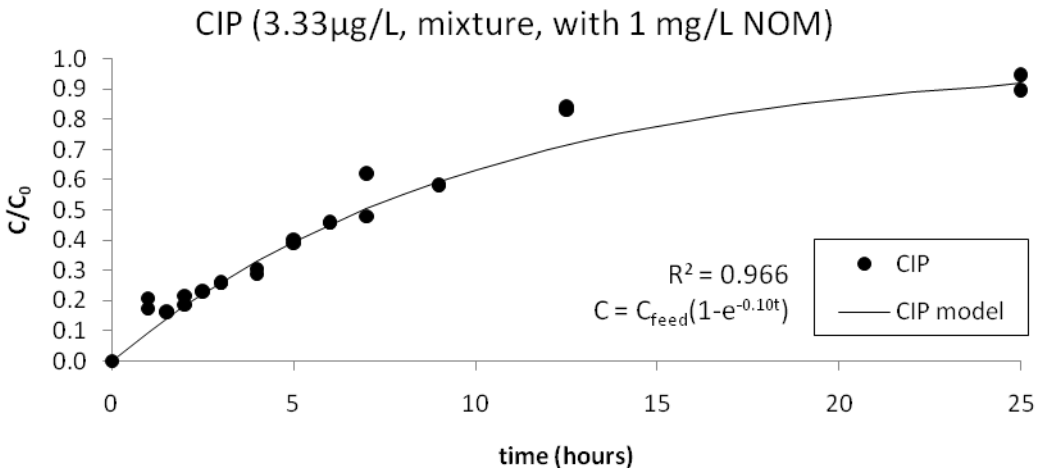


Table F.21. Antibiotic Data—0.33 µg/L (alone)

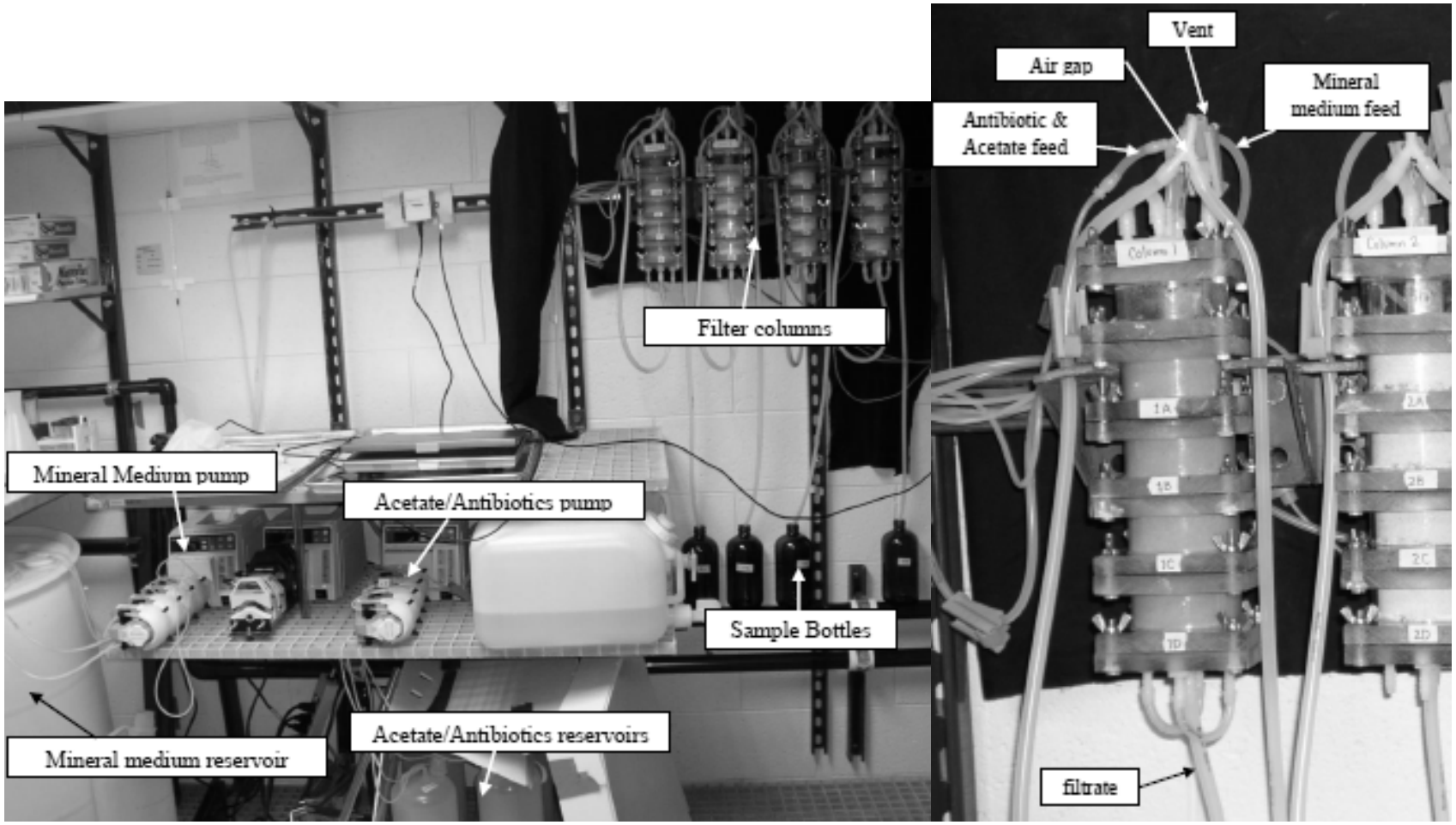
CIP	
feed cond	3.33 mixture w/ 1 mg/L NOM
date	29-Nov
k (hr^{-1})	0.1
R^2	0.966
Co	3.33
b (Co model)	1.18

raw data			model data		
t	C	C/Co	t	C	C/Co
0	0.00	0.00	0	0.00	0.00
0	0.00	0.00	1	0.11	0.10
1	0.21	0.17	2	0.21	0.18
1	0.24	0.21	3	0.31	0.26
1.5	0.19	0.16	4	0.39	0.33
1.5	0.20	0.17	5	0.46	0.39
2	0.25	0.22	6	0.53	0.45
2	0.22	0.19	7	0.59	0.50
2.5	0.27	0.23	8	0.65	0.55
2.5	0.27	0.23	9	0.70	0.59
3	0.31	0.26	10	0.75	0.63
3	0.30	0.26	11	0.79	0.67
4	0.36	0.31	12	0.82	0.70
4	0.34	0.29	13	0.86	0.73
5	0.47	0.40	14	0.89	0.75
5	0.46	0.39	15	0.92	0.78
6	0.54	0.46	16	0.94	0.80
6	0.54	0.46	17	0.96	0.82
7	0.73	0.62	18	0.98	0.83
7	0.57	0.48	19	1.00	0.85
9	0.69	0.58	20	1.02	0.86
9	0.68	0.58	21	1.04	0.88
12.5	0.99	0.84	22	1.05	0.89
12.5	0.98	0.83	23	1.06	0.90
25	1.06	0.90	24	1.07	0.91
25	1.12	0.95	25	1.08	0.92

Figure F.21. Effluent Antibiotic Concentration (C/C_0) versus time. CIP (0.33 µg/L, alone)



Appendix G. Labeled Photograph of Experimental Apparatus used for Filter Column Experiments



Appendix H. ARISA data for column studies (high concentration runs) from objective 3 experiments.

Table H.1 ARISA data for "high concentration" run: fragment lengths 171-520

Key:

3.33	experiment that considered antibiotic exposure @ 3.33 µg/L
0.33	experiment that considered antibiotic exposure @ 3.33 µg/L
A	column exposed to antibiotics
C	control--no antibiotics
0	sample collected at water-sand interface
5	sample collected at 5-cm depth
2	sample collected after 2-weeks operation
4	sample collected after 4-weeks operation
6	sample collected after 6-weeks operation

conc. (µg/L)	feed	depth (cm)	time (weeks)	replicate	fragment length																				
					171	184	220	261	270	280	305	349	396	431	453	455	461	463	465	468	470	475	518	520
3.33	C	0	2	1													5.5			8.0				
				2											1.7		3.7			1.0			1.0	
	A			1											10.6		3.4					1.1		
				2											1.2		7.9							
	C	5		1													14.1	1.3	4.6	23.1				
				2					1.4	1.3					1.5		12.5	1.3	4.9	12.8				
	A			1											9.6		7.3		2.6	7.6			2.0	
				2											17.8		3.8		3.0				2.8	
	C	0	4	1											4.9		3.3		1.6	3.2			3.8	
				2											9.0		5.6		1.6			1.4	3.2	1.6
	A			1	1.3	1.0	1.9					2.4		1.0	2.0		10.0	1.5	21.7	13.5		1.3		
				2											10.2	1.7	10.7		1.0				4.2	
	C	5		1											8.5		8.5			9.4				
				2											3.6		3.1			1.8				
	A			1				1.0							15.3		2.4							
				2											2.6		4.0			1.1				
	C	0	6	1					1.3			1.5			5.6		3.0	2.1		2.8			1.4	
				2											8.7		9.7		2.8	4.7			1.3	
	A			1				2.2						1.0	36.5		9.6			1.2			1.1	1.2
				2				1.2						1.0	22.5	2.0	11.6		1.1	1.2				
	C	5		1				1.2		1.8		1.1			12.8		1.8		1.0	1.0			4.3	
				2									1.3		8.6		5.3		1.6				3.1	
	A			1			1.1	1.4				2.6			9.8	2.2	4.5	2.7	3.6	2.0	2.3			
				2				1.8			2.3				32.4		5.4							

Table H.2 ARISA data for "high concentration" run: fragment lengths 524-606

Key:

3.33	experiment that considered antibiotic exposure @ 3.33 µg/L
0.33	experiment that considered antibiotic exposure @ 3.33 µg/L
A	column exposed to antibiotics
C	control--no antibiotics
0	sample collected at water-sand interface
5	sample collected at 5-cm depth
2	sample collected after 2-weeks operation
4	sample collected after 4-weeks operation
6	sample collected after 6-weeks operation

conc. (µg/L)	feed	depth (cm)	time (weeks)	replicate	fragment length																		
					524	530	534	537	538	541	545	548	553	556	565	577	580	583	587	590	591	594	606
3.33	C	0	2	1	1.1			1.2								4.7							
				2												1.0			2.8				
	A			1				2.2								2.2							
				2				2.8											3.4	5.1			
	C	5		1						2.3						2.1						1.1	
				2						5.1						2.3			1.0			1.7	1.4
	A			1				2.1							2.9	4.1				2.1			
				2				3.3							1.2					8.1			
	C	0	4	1									1.8	1.5		8.5		1.7		2.9			
				2							1.5	2.6				10.0		1.4		9.9			
	A			1				9.6		5.3							1.0			1.6			
				2		1.4	1.3	10.3							4.1					1.6			
	C	5		1		1.4												1.2					
				2																			
	A			1					1.9												1.3		
				2						2.4													
	C	0	6	1						15.1								1.3				5.7	1.2
				2				12.4		1.5						4.9							
	A			1				5.2			1.8					1.1							
				2				16.3								2.0							
	C	5		1						2.9			2.4	3.2		2.2							
				2		2.1		17.7					1.7	1.8	1.2	1.3							
	A			1		1.7		4.6		16.9	18.3					1.5							
				2				29.8							1.1								

Table H.3 ARISA data for "high concentration" run: fragment lengths 616-690

Key:

3.33	experiment that considered antibiotic exposure @ 3.33 µg/L
0.33	experiment that considered antibiotic exposure @ 3.33 µg/L
A	column exposed to antibiotics
C	control--no antibiotics
0	sample collected at water-sand interface
5	sample collected at 5-cm depth
2	sample collected after 2-weeks operation
4	sample collected after 4-weeks operation
6	sample collected after 6-weeks operation

conc. (µg/L)	feed	depth (cm)	time (weeks)	replicate	fragment length																
					616	621	625	627	629	630	634	637	642	648	650	668	672	673	677	679	688	690
3.33	C	0	2	1			3.1		16.9				2.2	1.6				1.1			7.6	
				2			7.8		10.6				3.2								1.6	1.1
	A			1			18.3		2.1				5.1	7.1	2.1			1.1			3.7	
				2			6.6		18.8			1.1		6.1								1.9
	C	5		1			4.2					4.1		1.4				5.9				5.5
				2		1.1	1.3		1.2			5.3						2.4				7.7
	A			1			8.7					2.4		4.2		1.8		2.5				2.8
				2			9.5		1.6			4.4		2.4		11.1		1.9				0.9
	C	0	4	1			17.9					7.5		2.2				1.2				5.4
				2			2.6	6.2				7.2		3.3				1.0				3.3
	A			1			1.4					4.3							1.0			2.6
				2			15.3					3.8		2.4				1.6				1.8
	C	5		1	1.4		2.0		5.9				5.4					3.4				3.1
				2			8.4		19.6				3.8					1.0				3.1
	A			1			2.8		23.0				9.7					2.1				2.4
				2		1.0	2.0		3.7													45.9
	C	0	6	1		3.7	4.6		1.8			3.4						2.5			1.0	11.3
				2			2.5			2.4		2.2						3.7				7.9
	A			1			5.1			1.4			1.3					9.1				
				2			3.6		2.6			2.2						5.8				9.1
	C	5		1			2.0					2.7						1.3				26.8
				2		1.1				1.7		2.3						1.9				21.3
	A			1		1.2	1.1			2.8		2.6						2.2			1.7	1.1
				2														5.6				

Table H.4 ARISA data for "high concentration" run: fragment lengths 696-794)

Key:

3.33	experiment that considered antibiotic exposure @ 3.33 µg/L
0.33	experiment that considered antibiotic exposure @ 3.33 µg/L
A	column exposed to antibiotics
C	control--no antibiotics
0	sample collected at water-sand interface
5	sample collected at 5-cm depth
2	sample collected after 2-weeks operation
4	sample collected after 4-weeks operation
6	sample collected after 6-weeks operation

conc. (µg/L)	feed	depth (cm)	time (weeks)	replicate	fragment length																			
					696	700	704	707	714	717	722	729	741	744	750	756	757	760	767	770	775	785	790	794
3.33	C	0	2	1			9.4	1.1	12.3		1.2	2.2	2.8			1.2			2.7					
				2			16.3	1.9	22.4		2.3					2.4			2.7					
	A			1			11.2						1.6			4.8			2.1				1.3	
				2				23.6		1.4														
	C	5		1						2.3			2.0	2.3										
				2						3.2			1.5	1.1										
	A			1							1.2			1.0		10.4	7.0							
				2							3.7						8.8							
	C	0	4	1						6.9			1.4	9.4						1.5				
				2						5.4			3.0				1.9							
	A			1							1.3													
				2							1.8				6.7		9.2							
	C	5		1				6.1	1.4	15.1				7.3	1.8					3.3				
				2				6.3	1.2	15.3		1.3		14.8										
	A			1				15.8		1.1				5.4			1.4							
				2				13.7		2.7				2.1				1.8		1.0				
	C	0	6	1					1.4				1.0	1.7										
				2		3.1				2.1	1.4		8.4									1.0		
	A			1				1.1																
				2				1.0					1.8				2.2							
	C	5		1					2.9				2.3											
				2		4.3						1.2	2.1											
	A			1	1.0				1.7		1.1	1.8												
				2													2.9							

Appendix I. ARISA data for column studies (low concentration runs) from objective 3 experiments.

Table I.1 ARISA data for "low concentration" run: fragment lengths 171-520)

Key:

3.33	experiment that considered antibiotic exposure @ 3.33 µg/L
0.33	experiment that considered antibiotic exposure @ 3.33 µg/L
A	column exposed to antibiotics
C	control--no antibiotics
0	sample collected at water-sand interface
5	sample collected at 5-cm depth
2	sample collected after 2-weeks operation
4	sample collected after 4-weeks operation
6	sample collected after 6-weeks operation

conc. (µg/L)	feed	depth (cm)	time (weeks)	replicate	fragment length																				
					171	184	220	261	270	280	305	349	396	431	453	455	461	463	465	468	470	475	518	520
0.33	C	0	2	1											1.9		7.5			12.6				
				2											10.5				19.9					
	A			1											9.1		2.8		2.5					
				2											4.3		1.9			3.0				
	C	5		1											4.5		24.5	3.6		4.5				
				2											9.1		26.9	3.4		3.4				1.4
	A			1											2.4		23.4	2.8						1.0
				2											5.9		8.0			1.5				
	C	0	4	1											5.1		12.7	1.0	18.0	18.4				
				2											12.7		6.4		2.7	3.2				
	A			1											32.1				1.1				1.6	
				2											1.3		16.4	1.4	4.2	26.5			1.7	
	C	5		1											3.6		4.0		2.8				1.3	4.5
				2											7.5		6.1	1.0		1.1				1.9
	A			1											8.7	2.7	23.4	2.5					1.0	1.4
				2											5.2		12.3	1.3		2.4				
	C	0	6	1											4.3		9.1		2.2	14.5			3.0	
				2				1.0							29.2		6.1		1.4	1.6			5.4	
	A			1				1.2						1.0	27.5	19.6			1.6				1.3	
				2											2.9		10.7		1.7	20.5			5.9	
	C	5		1											1.4		1.0						11.1	1.4
				2				1.1							25.4	1.9	13.2			1.4			2.7	
	A			1											19.6		11.3		1.2				4.1	1.0
				2											7.1		6.7			1.7			2.7	

Table I.2 ARISA data for "low concentration" run: fragment lengths 524-605)

Key:

3.33	experiment that considered antibiotic exposure @ 3.33 µg/L
0.33	experiment that considered antibiotic exposure @ 3.33 µg/L
A	column exposed to antibiotics
C	control--no antibiotics
0	sample collected at water-sand interface
5	sample collected at 5-cm depth
2	sample collected after 2-weeks operation
4	sample collected after 4-weeks operation
6	sample collected after 6-weeks operation

conc. (µg/L)	feed	depth (cm)	time (weeks)	replicate	fragment length																		
					524	530	534	537	538	541	545	548	553	556	565	577	580	583	587	590	591	594	606
0.33	C	0	2	1				3.9								1.6							
				2	7.1																		
	A			1				1.6								1.1							
				2	8.1			1.0		1.4									7.6				
	C	5		1				1.5															
				2	3.4										4.2								
	A			1											1.5								
				2	52.3														1.2				
	C	0	4	1				2.4												3.3			
				2				6.4								1.4				5.8			
	A			1				4.4												6.6			
				2				2.8							1.9	1.0				1.9			
	C	5		1				2.8			1.0					5.7							
				2						1.3						2.2							
	A			1				4.1								1.7							
				2	4.7			4.1							1.3	1.2							
	C	0	6	1				5.7							1.4					3.5			
				2				8.3							3.4	1.4				1.6			
	A			1				5.2							1.8					2.5			
				2				10.2							1.7	1.1							
	C	5		1				16.0		2.6						3.3							
				2	1.5		1.3	6.2								3.8							
	A			1				5.3								1.5							
				2	1.6			15.4			2.3												

Table I.3 ARISA data for "low concentration"run : fragment lengths 616-690)

Key:

3.33	experiment that considered antibiotic exposure @ 3.33 µg/L
0.33	experiment that considered antibiotic exposure @ 3.33 µg/L
A	column exposed to antibiotics
C	control--no antibiotics
0	sample collected at water-sand interface
5	sample collected at 5-cm depth
2	sample collected after 2-weeks operation
4	sample collected after 4-weeks operation
6	sample collected after 6-weeks operation

conc. (µg/L)	feed	depth (cm)	time (weeks)	replicate	fragment length																
					616	621	625	627	629	630	634	637	642	648	650	668	672	673	677	679	688	690
0.33	C	0	2	1			30.8						2.5					2.2				
				2					6.3				22.3	1.1								1.0
	A			1			30.7		3.5										1.0			
				2				8.8	8.0		1.0		16.0									1.3
	C	5		1			26.5						1.3		2.1		5.6	3.1				
				2									5.2		1.8		9.7					2.7
	A			1			37.2								1.2		4.3					2.5
				2									1.6					3.2				
	C	0	4	1			10.5					4.0			1.4			2.9				4.4
				2			2.4		1.8			2.0	16.3	1.8				2.5				3.1
	A			1			31.4							1.4				3.6				
				2			2.4					1.4	5.3					4.7				4.7
	C	5		1			10.1															33.9
				2									2.6		2.1		2.8					50.3
	A			1			25.4								1.4		4.9	2.8				
				2									1.2				1.6	1.9				41.5
	C	0	6	1			31.8										4.3					
				2			2.7					1.9	2.9				3.3					2.3
	A			1			14.4										7.4					
				2			8.0						1.3	1.0			5.1					1.2
	C	5		1			37.0															1.1
				2									4.1				7.4					6.3
	A			1			30.4										6.1					
				2													2.4					43.5

Table I.4 ARISA data for "low concentration" run: fragment lengths 696-794)

Key:

3.33	experiment that considered antibiotic exposure @ 3.33 µg/L
0.33	experiment that considered antibiotic exposure @ 3.33 µg/L
A	column exposed to antibiotics
C	control--no antibiotics
0	sample collected at water-sand interface
5	sample collected at 5-cm depth
2	sample collected after 2-weeks operation
4	sample collected after 4-weeks operation
6	sample collected after 6-weeks operation

conc. (µg/L)	feed	depth (cm)	time (weeks)	replicate	fragment length																		
					696	700	704	707	714	717	722	729	741	744	750	756	757	760	767	770	775	785	790	794
0.33	C	0	2	1						1.8			1.5				1.2		2.0	2.2				
				2	1.0					1.7							4.0		1.6	3.4				1.2
	A			1						1.3			2.3				2.7		4.4	10.7				
				2	1.5					6.4							2.9		2.7	1.2				1.9
	C	5		1					1.3								3.2							1.6
				2					1.0								2.0			5.2			1.4	1.1
	A			1									1.0							1.4				1.5
				2					3.1				1.3							1.0				1.5
	C	0	4	1	1.0																			
				2	3.1												1.3			6.4	1.3			
	A			1																				
				2	1.9												1.5							
	C	5		1					9.2												1.2			
				2					1.7															
	A			1																				1.3
				2																				1.7
	C	0	6	1							1.4													
				2	2.0						2.1									6.7				
	A			1							1.5													
				2	2.9						2.2						1.2				1.4			1.6
	C	5		1					5.2												1.5			
				2																				3.4
	A			1							1.1													
				2																				1.2

Appendix J. Additional Model Parameter Data: Antibiotic Retardation and Breakthrough in Biofiltration Systems.

model parameters	SSF	RBF	RGBF
porosity	0.4	0.3	0.4
bulk density (kg/L)	2.6	2.6	2.6
filtration rate (m/hr)	0.1	0.1	10
depth of biologically-active material (m)[A]	0.025	0.1	0.5
depth of "clean" media or aquifer material (m)	1	10	1
biologically-active material f_{oc} (g/g)	0.004[F]	0.004[H]	0.00025[I]
"clean" media or aquifer material f_{oc} (g/g)	0.0001[G]	0.0001[G]	0.0001[G]

antibiotic	class	K_{oc} (L/kg)	SSF		RBF		RGBF	
			R^J	Breakthrough (days)[K]	R^J	Breakthrough (days)[K]	R^J	Breakthrough (days)[K]
Ciprofloxacin	Fluoroquinolone	90,000[B]	2341, 56	**49.2** (24.4,24.8)	3181, 81	**468.0** (132.5, 335.5)	147, 60	**0.55** (0.31,0.26)
Erythromycin	Macrolide	6,000[B]	157, 5	**3.68** (1.64, 2.04)	213, 6	**35.1** (8.9,26.2)	11, 5	**<0.1**
Sulfamethoxazole	Sulfanomide	4,000[B]	105, 4	**2.59** (1.09,1.50)	142, 5	**15.4** (5.9,9.5)	**7.5, 4**	**<0.1**
Oxytetracycline	tetracycline	50,000[C]	1301, 34	**16.68** (8.14,8.54)	1768, 45	**118.1** (51.6,66.5)	82, 34	**0.31** (0.17,0.14)
Efrotomycin	lipoglycoside	1000[E]	4, 1	**0.75** (0.17,0.58)	5, 1	**4.44** (1.07,3.37)	1, 1	**<0.1**

[A]schmutzdecke for SSF, colmation zone for RBF; depth of biological penetration for RGF.
[B]Wunder *et al.,* (in press) . Reported K_{oc} values for biofilm.
[C]based on values from Rabolle and Spliid, 2000. K_{oc} values (high organic sand) ranging from 27790 to 93320 L/kg.
[D]based on values from Rabolle and Spliid, 2000. K_{oc} values (high organic sand) ranging from 38 to 56 L/kg
[E]based on values from Yeager and Halley, 1990. K_{oc} values (high organic sand) ranging from 580 to 1460 L/kg
biologically-active section and subsequent "clean" media, respectively.
[F]based on measurements of VS at water-media from this study: O.C. assumed to be 49% of VS (i.e., $C_5H_7O_2N$)
[G]based on measurements of VS in last filter segment from this study: O.C. assumed to be 49% of VS (i.e., $C_5H_7O_2N$)
[H]assumed to similar to SSF
[I]based on values from Fonseca *et al*. (2001)
[J]First number is for biological zone, second number is for subsequent filtration zone
[K]First number is total time to breakthrough in system. Parenthetical numbers are breakthrough times through biological zone and subsequent filtration zone.