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Azithromycin sorption and biodegradation in a simulated California river system

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HIGHLIGHTS

- Aerobic: degradation rate constant=0.0084 ± 0.0039 day^{-1}, DT_{50} = 82.52 ± 56.54 days.
- No anaerobic degradation. Based on extracted products <1% degraded over 150 days.
- Microbial growth was observed despite antibiotic addition and soil being autoclaved.
- Sorption and desorption coefficients were calculated.
- Transport will fluctuate based on soil-water saturation & bulk movement of sediment.

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ABSTRACT

Azithromycin (AZ) is a widely-used macrolide antibiotic that is continually deposited into natural waterways by sewage effluent. Though recognized as an emerging contaminant of concern, little is known about its fate and transport in aquatic systems. American River soils and water were used to determine degradation of AZ in microcosms simulating flooded (anaerobic) and non-flooded (aerobic) California watershed conditions. Under aerobic conditions the degradation rate constant (k=0.0084 ± 0.0039 day^{-1}) and DT_{50} (82.52 ± 56.54 days) were calculated, as AZ disappearance indicated potential degradation. However, based on concurrent product appearance, less than one percent of the parent degraded over 150 days. Throughout the experiment microbial growth was observed by culturing in tryptic soy broth despite antibiotic addition and soil being autoclaved. Sorption likely contributes to AZ recalcitrance, thus the soil-water partition coefficient (log K_d = 2.18 Lkg^{-1}), Freundlich sorption and desorption coefficients (log K_f = 1.90 ± 0.14 and log K_f^d = 2.51 ± 0.30, respectively), and organic-carbon-normalized distribution coefficient (log K_{oc} = 4.25 Lkg^{-1}) were also calculated. Based on these results, AZ degradation in aquatic systems will likely be very limited and transport will fluctuate based on the extent of soil-water saturation or bulk movement of sediment.

1. Introduction

Azithromycin (AZ, Fig. 1) is a semi-synthetic macrolide antibiotic (Bright et al, 1988). High potency and wide applicability make AZ useful in veterinary medicine and one of the most widely used human antibiotics, with over 50 million prescriptions in the United States in 2010 alone (Foulds et al., 1990; IMS Institute for Healthcare Informatics, 2011; CDC, 2013). Additionally, AZ holds a position on the World Health Organization's Model List of Essential Medications (WHO, 2013).

Due to limited absorption and metabolism plus inadequate means to prevent environmental release through sewage effluents, AZ is recognized by the U.S. Environmental Protection Agency as an emerging contaminant of concern (Foulds et al., 1990; EPA, 2009; Verlicchi et al., 2012). It has been shown to accumulate in non-target species (caddisfly larvae) living in contaminated waters, and has been linked to toxic effects including inhibition of p-glycoprotein (Kim et al., 1999; Asakuraa et al., 2004; Grabicova et al., 2014). Environmental antibiotic loads can modify native microbial communities and diversity thereby altering natural biogeochemical cycling, causing potentially detrimental effects on

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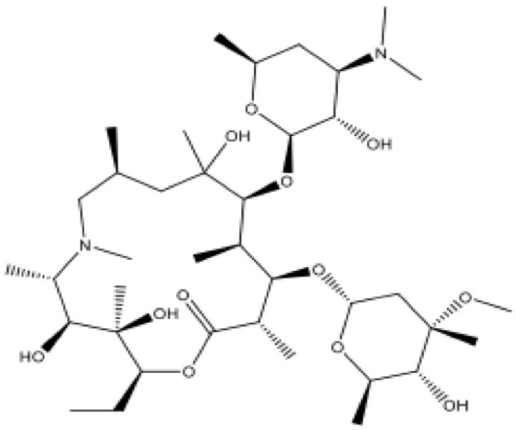

Water solubility: Estimated 2.73 mg L^{-1} (at 25 °C) (US EPA, 2012)

LogP (or Log K_{ow}) = 4.02 (McFarland et al., 1997)

pKa = 8.4 (at 25 °C) (McFarland et al., 1997)

Fig. 1. Azithromycin and some environmentally relevant properties(McFarland et al., 1997; US EPA, 2012).

agriculture as well as contributing to the growing worldwide antibiotic resistance epidemic (Alexy et al., 2004; Verlicchi et al., 2012; CDC, 2013).

Though identified worldwide in both fresh and sea water, as well as sediments, the fate of AZ is unclear (EPA, 2009; Jones-Lepp et al., 2012; Verlicchi et al., 2012). Experiments using sewage biosolid-soil mixtures resulted in AZ half-life estimations between 71 and 951 days; however, this may not be representative of degradation in aquatic systems, as microbial communities vastly differ between ecosystems (Sylvia et al., 2004; Walters et al., 2010; Gottschall et al., 2012). Structural analogs have also been shown to be relatively recalcitrant to biodegradation in closed bottle tests, but little is known about AZ degradation under standardized test conditions or aquatic systems impacted by sewage effluent (Alexy et al., 2004).

The health of freshwater systems in California directly impacts highly valued arable land, species diversity, and the Pacific Ocean. With AZ detected in aquatic systems throughout the state it is important to understand fate and transport of this efficacious antibiotic under typical watershed conditions (Jones-Lepp et al., 2012; Verlicchi et al., 2012). The American River is a representative freshwater system, providing for both agricultural and domestic uses, and ultimately feeding into the Sacramento River and San Francisco Bay (Shulters, 1982).

This investigation assessed potential AZ degradation and partitioning in a representative system. Degradation microcosms were constructed using water and soils collected from the American River under both flooded (anaerobic) and non-flooded (aerobic) soil conditions and incubated at 26 ± 1 °C. Degradation rates and half-lives were calculated and microbial growth in the presence of the antibiotic was monitored. Microcosms were evaluated for disappearance of the parent and concurrent appearance of both target and non-target degradation products. Also, soil sorption is characterized via calculation of soil-water partition coefficients for both AZ and its degradation products.

2. Materials and methods

2.1. Chemicals

Analytical grade azithromycin (Fluka, ≥ 95.0% purity) was purchased from Sigma-Aldrich (St. Louis, MO), while desclandose azithromycin (98.0% purity), 9a-N-desmethyl azithromycin, N′-desmethyl azithromycin, and azithromycin-d_3 were purchased from Toronto Research Chemicals (98.0% purity; Toronto, ON, Canada; Fig. 5). HPLC-grade water, acetonitrile (≥99.9% purity), and methanol (≥99.9% purity) were also purchased from Sigma-Aldrich, as was 5% dimethyldichlorosilane in toluene ("Sylon CT," Supelco). Ammonium hydroxide (ACS grade), calcium chloride dihydrate (Certified ACS) and formic acid (Optima, LC/MS grade, assay percent range ≥ 99.5%) were purchased from Fisher Scientific (Fisher Chemical, Chicago, IL), while tryptic soy broth (TSB) was obtained from MP Biomedicals (Solon, OH).

2.2. Soils

Soils were collected east of the American River Parkway — Howe Avenue River Access (Sacramento, CA; GPS coordinates 38° 33′ 36.66″ N: 121° 24′ 8.80″ W and 38° 33′ 33.61″ N: 121° 24′ 8.67″ W, respectively) in April 2016. This area had no known history of sewage introduction for more than 20 miles upstream. Several kilograms of sediment were collected from the top 0–10 cm layer of the river bank and an adjacent submerged area with slow water movement, passed through a 2-mm sieve and immediately incubated at 26 ± 1 °C. This temperature approximates Sacramento River Valley soil conditions (5–28 °C) when general soil microbial metabolic activity would be high due to warm conditions (Walters et al., 2010; Schindlbacher et al., 2011; Gottschall et al., 2012; USGS, 2016). Collection equipment was sterilized prior to use.

Organic carbon content and physical-chemical properties were determined by the UC Davis Analytical Laboratory (methods at anlab.ucdavis.edu). Submerged river soil was composed of less sand (79.5%) and clay (5%) than riverbank soil (84.5% and 6%, respectively), but greater amounts of silt and organic carbon (15.5% and 1.11% versus 9.5% and 0.87%, respectively). The pH of river and riverbank soils were 6.22 and 6.77, respectively.

2.3. Whole soil microcosm experiment

Aerobic microcosms were prepared with a 2.5 cm layer of riverbank soil (52.77 ± 9.28 g) in loosely-capped 250 mL amber glass jars. Moisture content was adjusted to approximately 50% water-holding capacity and maintained throughout the experiment using sterile HPLC water. Controls were triple-autoclaved (121 °C for 40 min on three consecutive days, incubated at 26 ± 1 °C between autoclaving) prior to the addition of 1 mg kg^{-1} (6.68×10^{-8} mol) AZ (in sterile HPLC water) at the start of the experiment (0 days; (Carter et al., 2007).

Flooded bottom soil was kept submerged throughout sieving and the addition of an approximate 2.5 cm soil layer (69.23 ± 13.66 g) to 250 mL amber jars. These anaerobic microcosms were transported under exclusion of oxygen with flood depth adjusted to 10 cm (approx. 200 mL) in the lab and tightly capped. Controls were prepared using sterile HPLC water and autoclaved soil. Relative redox potential (E_{hs}) and pH of flooded soil were measured throughout the experiment using pH and ORP meters (Hanna Instruments; Woonsocket, RI) to ensure both parameters were maintained. Collected samples and lab created controls developed comparable redox potentials prior to the addition of 1 mg kg^{-1} AZ (6.68×10^{-8} mol in sterile HPLC water).

Both autoclaved and non-autoclaved soils not spiked with AZ were extracted as blank controls. Aqueous controls were prepared using 200 ml sterile HPLC water and 1 mg L^{-1} AZ (2.67×10^{-7} mol in sterile HPLC water) in tightly-capped 250 mL amber glass jars. Both types of whole soil samples (n = 6), controls (n = 5), blanks