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Short Communication

Altered antibiotic tolerance in anaerobic digesters acclimated to triclosan or triclocarban

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HIGHLIGHTS

- Antimicrobials can alter anaerobic digester biomass tolerance to antibiotics.
- Triclocarban decreased tolerance of biomass to tetracycline (synergistic inhibition).
- Triclosan increased tolerance of biomass to ciprofloxacin (cross-resistance).

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ABSTRACT

Bench-scale anaerobic digesters were amended to elevated steady-state concentrations of triclosan (850 mg/kg) and triclocarban (150 mg/kg) using a synthetic feed. After more than 9 solids retention time (SRT) values of acclimatization, biomass from each digester (and a control digester that received no antimicrobials) was used to assess the toxicity of three antibiotics. Methane production rate was measured as a surrogate for activity in microcosms that received doses of antibiotics ranging from no-antibiotic to inhibitory concentrations. Biomass amended with triclocarban was more sensitive to tetracycline compared to the control indicating synergistic inhibitory effects between this antibiotic and triclocarban. In contrast, biomass amended with triclosan was able to tolerate statistically higher levels of ciprofloxacin indicating that triclosan can induce functional resistance to ciprofloxacin in an anaerobic digester community.

1. Introduction

Antibiotic resistance is a growing public health concern resulting in thousands of deaths every year (CDC, 2013). Antibiotic resistance is influenced and stimulated by many types of stressors including antibiotics, antimicrobials, and metals in a variety of environments (Alanis, 2005; McNamara et al., 2014; Carey and McNamara, 2015). Of particular concern is the development of cross-resistance whereby resistance to one stressor results in resistance to another stressor (Sefton, 2002). Calls to prudently prescribe antibiotics and minimize their use in agriculture stem in part from desire to quell promotion of cross-resistance and the corresponding spread of 'superbugs'.

Beyond prescription antibiotics, resistance to household antimicrobials has been documented, which yields concern that cross-resistance to clinically relevant antibiotics could develop from antimicrobial-Bacteria interaction. TCS and TCC are two household antimicrobial chemicals found in a range consumer products including antibacterial soaps. Cross-resistance to antibiotics stimulated by TCS has been investigated and discovered in many pathogenic bacteria (Giuliano and Rybak, 2015; Saleh et al., 2011). Although less investigated for its impact on cross-resistance to pathogens, similar concerns for TCC arise (Carey et al., 2016a; Chalew and Halden, 2009). The majority of cross-resistance studies have been conducted on pure-cultures, and research documenting cross-resistance in mixed communities is lacking.

The high concentrations of TCC and TCS in municipal wastewater and anaerobic digesters pose a particular concern because of

Abbreviations: CDC, Center for Disease Control; TCS, triclosan; TCC, triclocarban; USEPA, United States Environmental Protection Agency; IC_{50}, concentration that inhibits 50% of methane production; COD, chemical oxygen demand; SRT, solid retention time; ATA, anaerobic toxicity assay; pATA, prokaryotic anaerobic toxicity assay; K_{ow}, octanol water partition coefficient; VFA, volatile fatty acid.

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their interaction with a rich and diverse community of Bacteria. TCC and TCS were the most abundant pharmaceuticals (mean concentration of 39,433 μg/kg and 16,097 μg/kg respectively) found in US wastewater biosolids out of 145 chemicals surveyed (USEPA, 2009). Digesters contain a sub inhibitory mixture of antimicrobials and antibiotics that renders development of cross-resistance a strong possibility. Resistance development in biosolids is suspected to have a critical role in antibiotic resistance in terms of public health (Munir et al., 2011; Pruden, 2013; Ju et al., 2016).

The objective of this research was to determine if long-term TCC or TCS exposure in anaerobic digesters impacted functional resistance (as measured by methane production) to antibiotics. Specifically, the toxicity level (IC_{50}) of tetracycline, ciprofloxacin, and chloramphenicol was measured in anaerobic microcosms to determine if exposure to these antimicrobials made microbial communities more or less susceptible to antibiotics. These antibiotics were chosen because they are all separate classes of antibiotics, have variable water chemistries, and are associated with cross-resistance to TCS (see Table 1). Furthermore, most cross-resistance mechanisms that have been identified are efflux pumps that have been upregulated (See Table 1), although it is possible horizontally transferred genes could be responsible for cross resistance.

2. Materials and methods

2.1. Acclimatizing mother reactors to TCC or TCS

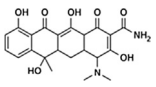
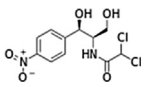
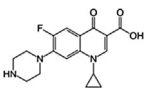
Three mother digesters were established as a biomass source for testing antibiotic toxicity against antimicrobial-acclimatized anaerobic biomass: a control digester, a TCS-amended digester, and a TCC-amended digester. These digesters are referred to as 'mother digesters' throughout this manuscript because the biomass from these digesters was used for inoculum for the experiments that tested antibiotic toxicity. Biomass from these digesters was used to determine the concentration of antibiotics required to inhibit 50% of methane production during batch methanogenic assays with each amended biomass. Each mother digester had 4 L of working volume and was seeded with biomass from a full-scale mesophilic anaerobic digester at South Shore Wastewater reclamation facility (Oak Creek, WI). Biomass from this facility was previously measured to have TCC and TCS concentrations of approximately 30 mg/kg for both antimicrobials in March of 2014 (Carey et al., 2016a, 2016b). The solid retention time of the mother digesters was 15 days and each digester was given 6 g of ground and sieved (40 mesh) dog food daily (1.8 g COD/L-d) in nutrient medium to simulate primary sludge. TCC and TCS was added to each feed to achieve steady state concentrations of 150 mg TCC/kg solids and 850 mg TCS/kg solids in the method outlined previously (control had no TCC or TCS addition) (Carey et al., 2016a). Digesters were operated for a total of 210 days. Qausi steady-state operation was established over the first 140 days (>9 SRT values), and biomass was used for toxicity testing over the remaining 70 days.

2.2. Prokaryotic anaerobic toxicity assay (pATA)

Anaerobic toxicity assay (ATA) style tests were performed to test the toxicity of three antibiotics (Stuckey et al., 1980). Methane production is measured as a surrogate for activity at different doses of a toxicant during batch tests. The experiments performed here differ from traditional ATAs in that a more complex feed carbon source was utilized (dog food) instead of acetate. Dog food was used because degradation to produce methane flows through all trophic groups (Bacterial and Archaeal) in an anaerobic digester. Although Archaea are widely thought to be immune to the actions of the inhibitors used in this experiment (i.e., antibiotics), chloramphenicol has been shown to inhibit Archaea (Hilpert et al., 1981), and the potential for minor or major inhibition in these experiments remains a possibility. Given that trophic groups from Bacteria or Archaea were potentially inhibited (as opposed to only Archaea in a traditional ATA), the modified assays that are performed in this work are referred to as "prokaryotic anaerobic toxicity assays" (pATAs), as prokaryotic refers to Bacteria and Archaea.

Prior to performing a pATA, waste biomass was collected from the mother digesters over a five day period. The biomass was allowed to degas for an additional 3 days before testing. For a given pATA test, a constant volume of biomass (50 mL) and a constant COD load (3.5 g COD/L) was employed for each glass serum bottle (160 mL) reactor. For these experiments, seven antibiotic doses were used in triplicate to span several orders of magnitude. The

Table 1
Antibiotic properties.

	Tetracycline	Chloramphenicol	Ciprofloxacin
Structure			
Class	Polyketide	Other[a]	Fluoroquinolone
Log K_{ow}	−1.37	1.14	0.28
Water solubility	231 mg/L @ 25 °C	2500 mg/L @ 25 °C	30,000 mg/L @20 °C
pKa	3.3	5.5	6.1
Concentration in biosolids[b]	1914 μg/kg	NA[c]	6858 μg/kg
TCS link to cross-resistance	Cross-resistance to tetracycline forms from TCS exposure in pathogens (eg. *E. Coli*, *P. aeruginosa*)	Mechanisms associated with TCS resistance are also associated with ciprofloxacin resistance	Chloramphenicol resistance is stimulated by exposure to TCS in pathogenic bacteria (eg. *S. Maltophilia*, *S. enterica serovar Typhimurium*)
Genes associated with resistance to TCS and antibiotic	acrAB (efflux), smeDEF (efflux), nfxB (efflux)	acrAB (efflux)	acrAB (efflux) nfxB (efflux)
References	Braoudaki and Hilton, 2004; Chuanchuen et al., 2001; Kappell et al., 2015; Sanchez et al., 2005; Karatzas et al., 2007	Piddock, 2006	Birosová and Mikulásová, 2009; Braoudaki and Hilton, 2004; Karatzas et al., 2007; Sanchez et al., 2005; Chuanchuen et al., 2001

[a] Chloramphenicol inhibits bacteria uniquely, but is somewhat related to macrolides.
[b] Mean concentration found in 72 treatment plants by McClellan and Halden, 2010.
[c] Chloramphenicol was not analyzed by McClellan and Halden, 2010.