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Impacts of solids retention time on trace organic compound attenuation and bacterial resistance to trimethoprim and sulfamethoxazole

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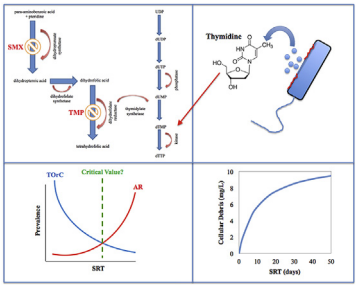
HIGHLIGHTS

- Environmental reservoirs of thymine/thymidine reduce trimethoprim efficacy.
- Longer SRTs increase single and multi-drug resistance in wastewater.
- Lower temperatures decrease antibiotic resistance in wastewater.

GRAPHICAL ABSTRACT



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ABSTRACT

Bacteria can grow in the presence of trimethoprim and sulfamethoxazole by expressing antibiotic resistance genes or by acquiring thymine or thymidine from environmental reservoirs to facilitate DNA synthesis. The purpose of this study was to evaluate whether activated sludge serves as a reservoir for thymine or thymidine, potentially impacting the quantification of antibiotic resistant bacteria. This study also assessed the impacts of varying solids retention time (SRT) on trimethoprim and sulfamethoxazole removal during wastewater treatment and single and multi-drug resistance. When assayed in the presence of the antibiotics at standard clinical concentrations, up to 40% increases in the relative prevalence of resistant bacteria were observed with (1) samples manually augmented with reagent-grade thymidine, (2) samples manually augmented with sonicated biomass (i.e., cell lysate), (3) samples manually augmented with activated sludge filtrate, and (4) activated sludge samples collected from reactors with longer SRTs. These observations suggest that longer SRTs may select for antibiotic resistant bacteria and/or result in false positives for antibiotic resistance due to higher concentrations of free thymine, thymidine, or other extracellular constituents.

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List of acronyms

AR	antibiotic resistance
ARB	antibiotic resistant bacteria
ARG	antibiotic resistance gene
CFU	colony forming unit
DNA	deoxyribonucleic acid
MH	Mueller Hinton
MIC	minimum inhibitory concentration
MLSS	mixed liquor suspended solids
MLVSS	mixed liquor volatile suspended solids
PSF	post-sonication filtrate
RAS	return activated sludge
RNA	ribonucleic acid
SBR	sequencing batch reactor
sCOD	soluble chemical oxygen demand
SMX	sulfamethoxazole
SRT	solids retention time
THY	thymidine
TMP	trimethoprim
TOC	total organic carbon
TOrC	trace organic compound
WAS	waste activated sludge
WWTP	wastewater treatment plant

1. Introduction

Wastewater treatment plants (WWTPs) play an important role in protecting human and environmental health from wastewater-derived pollution, but they are also considered significant reservoirs of antibiotic resistance (AR) (Novo and Manaia, 2010). Previous studies have highlighted the selective pressure exerted on bacteria in wastewater matrices and the impact of wastewater treatment on antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) (Schwartz et al., 2003; Luo et al., 2014; Su et al., 2014; Guo et al., 2015; Zhang et al., 2015).

Engineered biological treatment systems in WWTPs are intended to maximize bacterial activity and growth. Coupled with continuous exposure to trace levels of antibiotics found in municipal wastewater, these systems have the potential to increase concentrations of ARB by promoting horizontal gene transfer (Wang and Schaffner, 2011; Martínez, 2008) and/or inhibiting antibiotic susceptible bacteria (Lopatkin et al., 2016). However, there is still no consensus as to whether WWTPs truly contribute to the proliferation of AR. Some studies suggest that WWTPs achieve significant reductions in ARB (Guo et al., 2015; Huang et al., 2012) and ARGs (Luo et al., 2014), while other research indicates that WWTPs serve as major contributors of both (Kim et al., 2010; Luo et al., 2014). Inconsistencies may stem from temporal variability or differences in influent wastewater quality, treatment technologies and/or operational conditions, or methodologies for the detection of ARB and ARGs. Therefore, additional studies are needed to further elucidate the complex mechanisms influencing AR prevalence in wastewater.

One of the critical operational parameters for biological wastewater treatment, specifically the activated sludge process, is solids retention time (SRT). Potentially due to more abundant biomass and/or shifts in microbial community structure, longer SRTs have been correlated with lower total organic carbon (TOC) (Leu et al., 2012) and trace organic compound (TOrC) concentrations after biological wastewater treatment (Clara et al., 2005; Oppenheimer et al., 2007; Suarez et al., 2010; Melcer and Klecka, 2011; Salveson et al., 2012; Gerrity et al., 2013; Vuono et al., 2016). Gerrity et al. (2013) and Vuono et al. (2016) determined that lower effluent concentrations of the antibiotics sulfamethoxazole (SMX) and trimethoprim (TMP) could be achieved with longer SRTs in full-scale activated sludge and membrane bioreactor processes, respectively. However, studies that directly evaluate the relationship between SRT and the prevalence of ARB and/or ARGs are limited in the peer-reviewed literature (Kim et al., 2010). Therefore, additional studies are needed to assess the role of such operational conditions on AR occurrence, proliferation, and mitigation.

The current study addresses this knowledge gap by focusing on occurrence and resistance to the antibiotics TMP and SMX in activated sludge systems with varying SRTs. TMP and SMX cause bacteriostatic disruption of nucleotide synthesis for both Gram positive and Gram negative bacteria (Bushby and Hitchings, 1968; Snyder et al., 2013). The corresponding pathways are summarized in Fig. S1 in the Supplementary Information. Bacteria may be able to bypass these critical steps for DNA synthesis by obtaining thymine or thymidine from their environment. Amyes and Smith (1974) discovered that secondary reservoirs of thymine/thymidine, which are present at varying concentrations in different types of growth media, reduce the antibacterial efficacy of TMP. For this reason, TMP sensitivity tests require nutrient media with limited thymine/thymidine content [e.g., Mueller Hinton (MH) agar]. However, free thymine/thymidine in environmental samples may result in overestimation of AR prevalence unless bacteria are separated from their matrix before assay. For example, longer SRTs lead to higher rates of cell death and decay (Metcalf and Eddy, 2014), which can potentially generate an environmental reservoir of thymine/thymidine as the intracellular components are released from the cells.

This research explores the potential impact of thymine/thymidine and other extracellular constituents present in activated sludge on the quantification of culture-based antibiotic resistance. Specifically, this research assesses the potential for type I error (i.e., false positives) when quantifying bacterial resistance to SMX and TMP in wastewater matrices. Experiments were performed to evaluate the impacts of secondary thymidine reservoirs, cellular debris, and SRT on single- and multi-drug resistance. TOrC attenuation, specifically TMP, SMX, and the indicator compound atenolol, was also monitored during the SRT experiments.

2. Materials and methods

2.1. Description of laboratory-scale sequencing batch reactors

The laboratory-scale activated sludge process consisted of four parallel SBRs (Fig. S2) fed with primary effluent from a full-scale municipal WWTP in Las Vegas, Nevada. The acrylic SBRs had a total volume of 8 L and a working volume of 4 L after accounting for the volume of settled solids. Automation of the SBRs was achieved with a series of multi-station outlet timers, a peristaltic pump, electric actuated ball valves, and solenoid valves, as described in the supporting text for Fig. S2.

The SBRs were initially seeded with return activated sludge