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# Behavior of tetracycline and macrolide antibiotics in activated sludge process and their subsequent removal during sludge reduction by ozone

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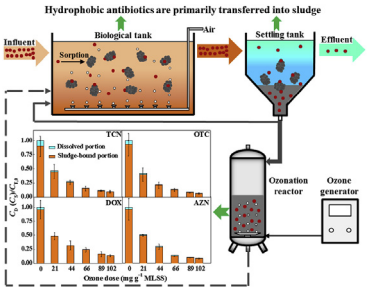
## HIGHLIGHTS

- TCN, OTC, DOX and AZN were primarily transferred into the sludge via sorption.
- The cations of antibiotics had higher sorption affinity to sludge than other species.
- 86.4–93.6% of antibiotics in sludge can be removed at 102 mg $O_3$ $g^{-1}$ MLSS and pH 7.2.
- Alkaline pH favored the elimination of antibiotics during sludge ozonation.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Ozonation is a promising means for the reduction of excess sludge in wastewater treatment plants. However, little information is available on the removal of antibiotics during sludge ozonation. Therefore, this study investigated first the behavior of four commonly-used hydrophobic antibiotics, including three tetracyclines (tetracycline, oxytetracycline, and doxycycline) and one macrolide (azithromycin) in activated sludge process and then their removal during sludge reduction by ozone. Results indicate that the studied antibiotics were primarily transferred into the solid phase of activated sludge via sorption, which was a reversible, spontaneous, and exothermic process governed by surface reactions. Sludge ozonation could effectively remove 86.4–93.6% of the antibiotics present in the sludge at an ozone dose of 102 mg per gram of mixed liquor suspended solids and pH 7.2. The removal of studied antibiotics mainly proceeded through desorption and subsequent oxidation. Increasing the initial pH from 5.0 to 9.5 obviously enhanced the antibiotic removal during sludge ozonation. This study demonstrated that the activated sludge process coupled with sludge ozonation can simultaneously reduce excess sludge and eliminate antibiotics.



## 1. Introduction

In recent years, antibiotics have been frequently detected in various environmental matrices such as water, soil, sediment, and

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biota (Gothwal and Shashidhar, 2015). The persistence of antibiotics in ecosystems could influence the evolution of microbial structure, thereby threatening the ecological health (Sharma et al., 2016). Moreover, the selection pressure originating from residual antibiotics on the environmental microbe could encourage the formation and spread of antibiotic-resistant bacteria and genes in a long term (Arias and Murray, 2009; Inyinbor et al., 2018). Antibiotic overuse and subsequent release has attracted the attention of environmental researchers and administrative departments in the world (Zhang et al., 2015). Many antibiotics are transported together with sewage into wastewater treatment plants (WWTPs), which were also an important point source of antibiotic contamination. The discharge of treated wastewater and excess sludge constitutes the release of antibiotics from the WWTPs to the natural environment. In recent years, a wide range of technologies (e.g., oxidation and advanced oxidation, adsorption, and membrane processes) have been applied to the elimination of antibiotics in the treated wastewater and other antibiotic-contaminated water (Benitez et al., 2011; Homem and Santos, 2011; Jiang et al., 2013). However, the elimination of antibiotics present in the sludge has not received sufficient attention.

As the annual production of excess sludge has been and will continue to increase in a foreseeable future, it is difficult to meet the more stringent standards and regulations for sludge disposal and land application relying only on the conventional sludge post-treatment processes (e.g., aerobic digestion, anaerobic digestion, and composting). Furthermore, the removal of many antibiotics (e.g., tetracyclines and macrolides) during sludge post-treatment processes was usually inefficient as a result of their resistance to biodegradation (Lindberg et al., 2006; Semblante et al., 2015), which will exacerbate the potential risk of excess sludge. Therefore, the great interest has been attracted on the in-situ sludge reduction based on various mechanisms (e.g., lysis-cryptic growth, uncoupling metabolism, and maintenance metabolism) (Guo et al., 2013). Among these processes, significant reduction of excess sludge could be achieved in the in-situ sludge ozone-reduction (Chu et al., 2009; Guo et al., 2013; Qiang et al., 2015). Considering the presence of electron-rich moieties (e.g., benzene ring and amino groups) in the molecular structures, many antibiotics could be efficiently degraded by ozone, as confirmed by previous studies performed in water phase (Homem and Santos, 2011; Jiang et al., 2013; Gomes et al., 2017). Thus, the in-situ sludge ozone-reduction process may also have the potential of eliminating many antibiotics present in the sludge. However, to date, little information is available on the elimination of antibiotics during sludge reduction by ozone (Carballa et al., 2007; Oncu and Balcioglu, 2013).

This study aims to elucidate the removal of four commonly-used and frequently-detected antibiotics, including three tetracyclines (doxycycline (DOX), oxytetracycline (OTC), and tetracycline (TCN)) and one macrolide (azithromycin (AZN)) during sludge ozonation. Since the antibiotic-sludge interactions could impact the degradation of antibiotics during ozonation, the behavior of antibiotics in the activated sludge process was first investigated by batch experiments. Thereafter, sludge ozonation experiments were conducted to investigate the removal of studied antibiotics at various ozone doses and initial pH values.

## 2. Materials and methods

### 2.1. Chemicals

The standards of AZN, DOX, OTC, and TCN were provided by Dr Ehrenstorfer GmbH (Augsburg, Germany). Azithromycin-$D_3$ (Toronto research Chemicals Inc., North York, ON, Canada) and demeclocycline (Dr Ehrenstorfer GmbH, Augsburg, Germany) were employed as internal standards. The purity of all the standards was ≥98%. The major physicochemical properties of studied antibiotics and internal standards are summarized in Table S1. HPLC-grade methanol and acetonitrile, formic acid (purity > 99%), and $NaN_3$ were obtained from Fisher Scientific (Geel, Belgium), Dikma Technologies, Inc. (Lake Forest, GA, USA), and Sigma-Aldrich (St. Louis, MO, USA), respectively. Other chemicals of analytical grade were purchased from Sinopharm Chemical Reagent Co., Ltd (Beijing, China). The stock solutions of studied antibiotics (100 mg $L^{-1}$ each) and internal standards (10 mg $L^{-1}$ each) were prepared in ultrapure water and 40% (v/v) methanol aqueous solution, respectively. Ultrapure water was produced from a Milli-Q system (Advantage A10, Millipore, USA).

### 2.2. Antibiotic behavior in activated sludge process

As shown in Table 1, five groups of batch experiments were conducted to clarify the behavior of studied antibiotics in the activated sludge process. The experiments were performed in 250-mL Erlenmeyer flasks wrapped with aluminum foil. Our preliminary experiments had shown that the sludge bioactivity was not influenced in the presence of studied antibiotics (100 μg $L^{-1}$ each), but was completely inhibited by $NaN_3$ (1.0 g $L^{-1}$). Hence, $NaN_3$ was used to inhibit the biodegradation of studied antibiotics in the activated sludge process. The contributions of different removal pathways were calculated based on the differences among Control A and Groups I and II. Groups III and IV were performed to determine the antibiotic sorption thermodynamics and the effect of initial pH on the sorption affinity, respectively.

Activated sludge was obtained from a lab-scale sequencing batch reactor (SBR) fed with synthetic wastewater (Text S1). To ensure the same matrix components for different experimental groups, the activated sludge was repeatedly centrifuged and washed with ultrapure water for three times, and then the remaining sludge was suspended in the synthetic wastewater. The treated sludge was spiked with the stock solution of studied antibiotics to achieve a desired initial concentration (Table 1). The dissolved oxygen (DO) concentrations under aerobic and anoxic conditions were maintained at 3.0–4.0 and < 0.5 mg $L^{-1}$, respectively. Temperature control and mixing were accomplished by a magnetic heated stirrer (Guohua, Changzhou, China), and the initial pH was adjusted by HCl and NaOH solutions. Slurry samples were taken with a syringe at pre-selected reaction time ($t$) (Table 1), and the antibiotic concentrations in the liquid phase ($C_D$) were analyzed.

### 2.3. Sludge ozonation

The excess sludge of SBR, with 4.6 g $L^{-1}$ mixed liquor suspended solids (MLSS), 4.2 g $L^{-1}$ mixed liquor volatile suspended solids (MLVSS), and pH 7.2, was used for sludge ozonation experiments. Groups V and VI were conducted to investigate the removal of studied antibiotics at different ozone doses and initial pH values (Table 1). Because the background concentrations were negligible, the studied antibiotics were externally spiked into the mix liquor to achieve the initial concentration of 800 μg $L^{-1}$. After magnetic stirring for 6 h, the initial pH of sludge was adjusted to a desired value (Table 1). After stirring for another 1 h, the sludge was subjected to ozonation in a semi-batch reaction system (sludge: batch addition; ozone: continuous supply) (Fig. S1) mainly consisting of an ozone generator (Tonglin, Beijing, China) and an ozonation reactor (cylindrical glass reactor, 1 L). Ozone gas was generated from pure oxygen, and bubbled into the ozonation reactor through a microporous aerator at a flow rate of 0.5 L $min^{-1}$. At the end of sludge ozonation (Table 1), the ozone stream was stopped, and the