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Short communication

# Enhanced removal of sulfamethoxazole with manganese-adapted aerobic biomass

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ABSTRACT

The antibiotic sulfamethoxazole (SMX) has been widely detected in water bodies. Better insights about its biodegradation pathways are required for developing removal processes and also for understanding its environmental fate. This study applied manganese carbonate to develop a biomass enriched with manganese oxidizing bacteria, in an aerated bioreactor which was fed with a synthetic secondary effluent and operated with a sequence batch mode. After two-month adaptation, an effective biodegradation of SMX occurred. The removal efficiency, ranging from 40% to 98%, was dependent on the feeding concentrations and hydraulic retention time. The removal was mainly due to the biological oxidation of manganese since it was completely repressed when a bacteriostatic agent (sodium azide) was added.



## 1. Introduction

Antibiotics have been widely detected in water bodies, which could bring potential risks to the ecosystem. For example, the antibiotic resistance of microorganisms can be developed and even spread through the biosphere (Baquero et al., 2008). The municipal effluent is one of the main sources of releasing antibiotics (Li et al., 2013). Therefore, removal technologies are of great concern in the management of antibiotic contamination.

Sulfamethoxazole (SMX), a broad spectrum sulfonamide, widely occurs in wastewater and surface water with concentrations up to 2000 ng $l^{-1}$ (Kümmerer, 2009). Its removal efficiency in wastewater treatment plants varies in a broad range (20–90%), possibly due to the different microbial communities (García-Galán et al., 2011; Verlicchi et al., 2012; Li et al., 2013). Tertiary-step treatments are on demand to further remove the emerging contaminants, including SMX. The popularly studied technologies include adsorption, membrane separation, and advanced oxidation (Altmann et al., 2014; Mansour et al., 2014; Phan et al., 2016). Biodegradation is advantageous over the chemical/physical processes with respect to the cost and easiness of operation; if only the contaminants can be oxidized by microorganisms, as is the case for the white rot fungi (Yang et al., 2013). However, the use of manganese oxidizing bacteria (MOB) in biodegradation processes has not been thoroughly investigated yet.

MOB can mediate the oxidation of $Mn^{2+}$ to its high-valence states. They extensively exist in natural water bodies and water treatment facilities. For instance, Cerrato et al. (2010) detected several strains of MOB in the sediment of surface water, on the filter materials of drinking water treatment, and in the water distribution pipes. Abu Hasan et al. (2012) isolated several MOB strains from activated sludge. In addition, researchers found that MOB are capable of degrading organic contaminants. Sabirova et al. (2008) observed that MOB can effectively degrade 17$\alpha$-ethinylestradiol (EE2) with the presence of $Mn^{2+}$. It is in accordance with the fact that manganese oxides have a high redox potential and both synthetic and biogenic MnOx can oxidize many organic and inorganic pollutants (Zhang et al., 2008; Hennebel et al., 2009; Zaman et al., 2009). Our recent study also obtained an effective removal (~90%) of SMX with a biofilter when $Mn^{2+}$ was spiked in the feeding and the removal was related to the active involvement of MOB (Zhang et al., 2015). However, as an attached growth bioreactor, the biofilter normally has a low volumetric loading and thus requires a large reactor volume for the treatment of wastewater. Furthermore, the construction cost would also be significantly higher than a suspended growth bioreactor. Furthermore, our preliminary study

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found that manganese carbonate was more effective than its ions for promoting the growth and biodegradation of MOB (Zhu et al., 2013).

Therefore, the objective of this study was to develop a MOB-enriched biomass in a suspended growth bioreactor for the removal of SMX. In this work we found that a constant and moderate supply of manganese carbonate to the reactor promoted the growth of MOB and SMX degradation.

## 2. Materials and methods

### 2.1. Chemicals and inoculum

Both SMX and manganese carbonate were of analytical grade and purchased from Sigma-Aldrich, Germany. Other chemicals were of reagent grade.

The back-washing sludge from a sand filter in a local drinking water treatment plant (Stolpe, Berlin, Germany) was taken as an inoculum, where the water resource contains iron and manganese (in the range of high μg $l^{-1}$ to low m $l^{-1}$).

### 2.2. Bioreactor and operation

A 5-L cylindrical reactor was filled with 4-L synthetic wastewater (DOC 10 mg $l^{-1}$, TN 9 m $l^{-1}$, pH 7.5) to simulate a secondary effluent. The composition can be found in a previous report (Zhang et al., 2015). The reactor was aerated at an air flow rate of 30 l $h^{-1}$ through a fine diffuser at its bottom. The aeration was sufficient to guarantee >7 mg $l^{-1}$ dissolved oxygen and to well mix the bioreactor as well.

The bioreactor was inoculated with the sludge mentioned above with a total solid content of 1 g $l^{-1}$ (measured as MLSS). 4 g $l^{-1}$ of manganese carbonate was added once at the beginning in order to promote the growth of MOB. The reactor was operated in a sequence batch mode with a volume exchange ratio of 50% at room temperature. The solid content was allowed to settle down for one hour before discharging water. The reactor was operated with different phases in terms of the hydraulic retention time (HRT, 2 d and 1 d) and the spiked influent concentration of SMX (2 mg $l^{-1}$, 20 μg $l^{-1}$, and 2 μg $l^{-1}$), as shown in Table 1.

Three samples were taken per week (once a week in the adaptation period). All samples were filtrated with 0.45 μm cellulose nitrate membrane and stored at 4 °C for the collective analysis. At the end of operation, a control test was conducted. 50 ml mixed liquor was taken from the reactor and transferred into a 300 ml shaked flask, containing 50 ml synthetic wastewater. 2 mg $l^{-1}$ SMX was spiked into the flask. The presence and absence of 50 mg $l^{-1}$ sodium azide was applied to evaluate the effect of bioactivity.

### 2.3. Analysis

SMX was analyzed with HPLC-UV for the high concentrations or HPLC-MS for the low concentrations. Both instruments and conditions were described in a previous report (Zhang et al., 2015). The analysis of dissolved organic carbon (DOC) and total Nitrogen (TN) were conducted with a DOC/TN analyzer (Analytik Jena multi N/C 3100). Manganese was analyzed by an atomic absorption spectroscopy (PerkinElmer PinAAcle 900 Z).

**Table 1**
The operational phases of bioreactor with different hydraulic retention time (HRT) and sulfamethoxazole (SMX) concentrations.

| Operation phase | Period | HRT | SMX |
|---|---|---|---|
| Adaptation | 65 d | 2 d | 2 mg $l^{-1}$ |
| Phase A | 38 d | 2 d | 2 mg $l^{-1}$ |
| Phase B | 35 d | 2 d | 20 μg $l^{-1}$ |
| Phase C | 23 d | 1 d | 20 μg $l^{-1}$ |
| Phase D | 44 d | 1 d | 2 μg $l^{-1}$ |

## 3. Results and discussion

### 3.1. Removal of SMX

Almost no removal of SMX was observed in the initial two months. The adaption time was comparable with the Mn-feeding biofilter packed with manganese oxides but shorter than one packed with natural zeolites (Zhang et al., 2015). The effective removal (>98%) of SMX appeared promptly between two adjacent sampling points (one week) and thereafter kept stable along the subsequent operation (Fig. 1). However, when SMX in the influent was dropped from 2 mg $l^{-1}$ down to 20 μg $l^{-1}$, the removal was decreased to 70% at the same HRT (2 d). Decreasing HRT to 1 d led to a declined removal (40%). However, when the influent SMX concentration was dropped from 20 μg $l^{-1}$ to 2 μg $l^{-1}$ at 1 d HRT, the removal climbed back to a median value of 57% and up to 100%, as shown in Fig. 1.

Profiling SMX depletion with time indicated that the adsorption contributed a limited portion to the removal. During Phase A much higher amounts of SMX were removed than in the subsequent phases (2 mg $l^{-1}$ vs. 20 μg $l^{-1}$ and 2 μg $l^{-1}$). If the high SMX removal in Phase A was due to adsorption, one would find a strong desorption effect in the followed operation and a higher level of SMX would appear in the effluent than the influent, which, however, did not occur in the experiment.

In order to clarify the biological effect, a control test was conducted with sodium azide as a bacteriostatic agent which can inhibit the biological metabolism, including the manganese oxidizing activity of MOB. The control test revealed that the bioactivity of MOB was essential for the removal of SMX. As shown in Fig. 2, the addition of sodium azide resulted in nearly no removal of SMX. It indicates that the inactivated biogenic MnOx did not

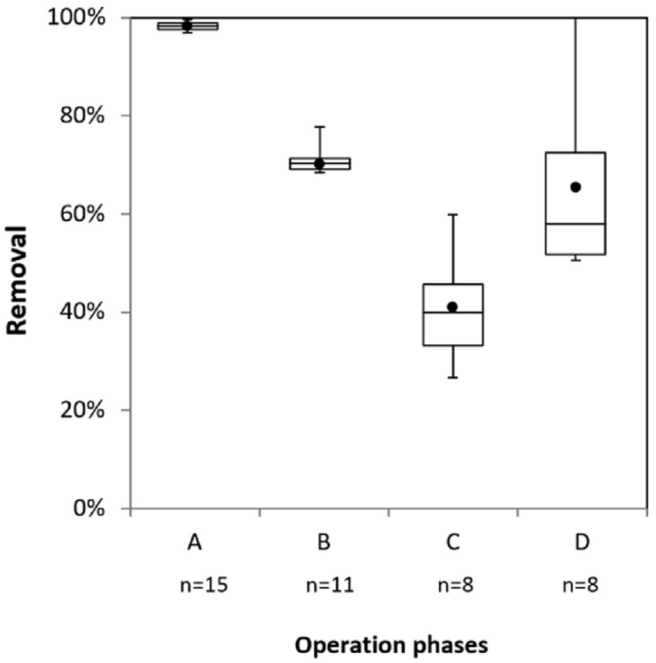


**Fig. 1.** Removal of SMX at different operation phases: A, HRT 2 d, SMX 2 mg $l^{-1}$; B, HRT 2 d, SMX 20 μg $l^{-1}$; C, HRT 1 d, SMX 20 μg $l^{-1}$; D, HRT 1 d, SMX 2 μg $l^{-1}$.