Supplementary Information

Significant effect of evaporation process on the reaction of sulfamethoxazole with manganese oxide

Jingchen Li¹, Lin Zhao¹, Ching-Hua Huang², Huichun Zhang³, Ruochun Zhang⁴, Samreen Elahi⁵, Peizhe Sun¹*

1 School of Environmental Science and Engineering, Tianjin University, Tianjin 300072, China
2 School of Civil and Environmental Engineering, Georgia Institute of Technology, Atlanta, Georgia 30332, United States
3 Department of Civil Engineering, Case Western Reserve University, Cleveland, Ohio 44106, United States
4 Institute of Surface-Earth System Science, Tianjin University, Tianjin 300072 China

Corresponding author: Peizhe Sun; Email: sunpeizhe@tju.edu.cn

Journal: Environmental Science & Technology

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Text S1. Chemicals

Manganese dioxide was purchased at 99.7% purity from Bodi Chemical industry, China. SMX was purchased from Melonepharma at 99.5% purity. SDM was purchased from Macklin at 98% purity. Methanol was purchased from Sigma-Aldrich at 99.9% purity. Sodium chloride was purchased from Yuanye at 99.5% purity. Mueller-Hinton Broth (MH (B)) was purchased from Solarbio at biological reagent. Other reagents used (e.g. sodium hydroxide, phosphoric acid, sodium acetate, acetic acid, manganese chloride, etc.) were all purchased from Kermel at analytical grade. All reagents were used without further purification. High purity deionized water was generated from a Millipore (Billerica, MA) Milli-Q® water purification system.

Text S2. Operation conditions for UPLC–MS/MS.

The elution solvents were 0.1% formic acid (A) and methanol (B) at the following gradients: initial, 5% B; 0-1 min, 25% B; 1-7 min, 35% B; 7-13 min, 95% B; 13-15 min, 95% B; 15–15.1 min, 5% B with a final hold for 3 min at a flow rate of 0.3 mL/min. The injection volume was 10 μL. The positive mode electrospray ionization (ESI+) over a mass scan range of 50-600 m/z was used for the mass spectrometry analysis. Operation conditions were as follows: source temperature of 550 °C; ion spray voltage of 5500 V; curtain gas at 35 psi; atomizing gas (GS 1) at 55 psi; dry gas (GS 2) at 50 psi, declustering potential (DP) of 50 V, and collision energy (CE) of 35±15 V.

Text S3. Toxicity Analysis Methods.

The antimicrobial properties of SMX and their transformation products were
tested using *Escherichia coli* (*E. coli*). Optical density (absorbance at 600 nm) were used as an indication of bacterial growth. All operations are carried out in a clean bench to ensure a sterile environment. The MH medium without bacteria was set as negative control for each experiment, to ensure no contamination of bacteria during the operations.

The specific steps:

1. Activated and amplification culture: 25 μL bacteria solution were transferred to 100 mL MH liquid medium and cultured for certain time in air bath shaker at 37 ℃, 120 r/min. The obtained bacteria solution in logarithmic growth phase can be used for the toxicity analysis experiment.

2. Growth inhibition of SMX standard solutions on *E. coli*: The antimicrobial property of SMX was measured at concentration of 0-20 μM. The inhibition was calculated from the following equation:

\[
\text{Inhibition(\%)} = (1 - \frac{\text{OD}_{600}^{(\text{SMX})}}{\text{OD}_{600}^{(\text{Control})}}) \times 100
\]

where \(\text{OD}_{600}^{(\text{SMX})}\) was the absorbance at 600 nm of the SMX standard solutions, and \(\text{OD}_{600}^{(\text{Control})}\) was the absorbance of blank solvent (without SMX) replacing the SMX standard solutions.

3. Growth inhibition of samples on *E. coli*: the samples were taken during reaction (SMX: 20 μM, \(\gamma\)-MnO\(_2\): 100 g/L in suspension or 10 g/L in evaporation), methanol were added to desorb the transformation products and remaining SMX on MnO\(_2\) by shaking at 200 rpm for 5 minutes. 2 mL sample was taken after rapidly pushing through a 0.22 μm membrane, and the methanol was replaced with deionized
water by evaporation. Then, 2 mL sample was mixed with 4 mL MH liquid medium and 6 μL bacteria solution obtained at step (1), and cultured for 8 h in water bath shaker at 37 °C, 170 r/min. The inhibition was calculated from the following equation:

\[
\text{Inhibition(\%)} = \left(1 - \frac{\text{OD}_{600}(\text{Sample})}{\text{OD}_{600}(\text{Control})}\right) \times 100
\]

where \(\text{OD}_{600}(\text{Sample})\) was the absorbance at 600 nm of the sample from solution after reaction, and \(\text{OD}_{600}(\text{Control})\) was the absorbance of blank solvent (without SMX) replacing the samples.

**Text S4. Calculation of model.**

The predicted SMX concentrations in the evaporation process was calculated as follows. The total SMX was calculated from the expression \(M_t' = C_t' V_t'\), and its change is shown in the following equations:

\[
dM_t'/dt = d(C_t'V_t')/dt = V_t' \times dC_t'/dt + C_t' \times dV_t'/dt \tag{1}
\]

We assumed that the degradation of SMX in the evaporation system follows the same first order kinetics as the one in the suspension system. Thus, \(dC_t'/dt\) can be expressed as follows:

\[
dC_t'/dt = -k_t C_t' \tag{2}
\]

Introducing \(V_t' = a \times t + b\) and Eq.2 to Eq. 1, we obtained Eqs. 3 and 4:

\[
dM_t'/dt = -k_t C_t' \times V_t' + \frac{d(a \times t + b)}{dt} \times C_t' = -k_t C_t' \times V_t' + aC_t'
\]

\[
= -k_t M_t' + aM_t'/V_t \tag{3}
\]

\[
\frac{(dM_t'/dt)/M_t}{M_t} = -k_t + a/V_t' \tag{4}
\]

Integrating both sides of Eq. 4:

\[
\ln \frac{M_{t2}'}{M_{t1}'} = \int_{t_1}^{t_2} (-k_t + a/V_t')dt \tag{5}
\]
where $M_t'$ is the predicted total molar mass of SMX at time $t$, in $\mu$mol; $C_t'$ is the predicted SMX concentration at a certain time without distinguishing the adsorbed amounts or the liquid phase, in $\mu$M; $V_t'$ is the reactant liquid volume at a certain time, calculated using $V_t' = a \times t + b$, in L; $k_t$ is the kinetic value at a certain time, calculated using $k_t = 2.72 \times 10^{-3} \times [\text{MnO}_2]$, in d$^{-1}$; $a$ is the fitting constant.

The molar change of SMX at any given time was calculated using Eq. 5, and the concentration of SMX at a given time ($C_t'$) was further predicted by combining the following relationship:

$$M_{t2}' / M_{t1}' = C_{t2}' V_{t2}' / C_{t1}' V_{t1}'$$  \hspace{1cm} (6)

To study the reaction in each time interval, another set of predicted values of SMX concentration was also calculated using the same methods, except for the replacement of the predicted $M_{t1}'$ and $C_{t1}'$ in Eq. 6 by the actual amount and concentration at $t_1$ (i.e. $M_{t1}$ and $C_{t1}$), respectively. In other words, each value was predicted based on the actual amount and concentration of SMX at a prior time point.

**Text S5. Experimental details.**

(a) change of MnO$_2$ activity

To investigate the change of MnO$_2$ activity during evaporation process, the MnO$_2$ was pre-treated by evaporating it in an acetate buffer at pH 6.3 for 3 days, without the addition of SMX. Then, the evaporated MnO$_2$ was dried and experiments in both suspension and evaporation systems were conducted.

(b) effect of Mn$^{2+}$

The effects of Mn$^{2+}$ on the reaction in suspension and on the evaporation system
at pH 6.3 were evaluated by adding excessive MnCl$_2$ (5 and 1 mM for suspension and evaporation, respectively). Reactors without MnCl$_2$ were also conducted as control experiments, with NaCl at same ionic strength (i.e. 5 and 1 mM for suspension and evaporation, respectively). Because the concentration after evaporation was excessively high if 5 mM MnCl$_2$ was used, smaller initial Mn$^{2+}$ concentration was used in the evaporation system than suspension system to increase the comparability between them. Moreover, the effect of different initial concentrations (10 μM to 10 mM) of additional MnCl$_2$ on the degradation of SMX in the evaporation process were also examined.

(c) effect of rainfall

To investigate the influence of rainfall on the degradation of SMX, water with the initial volume was replenished into the evaporation group every three days to simulate rainfall, and the evaporation process was repeated (i.e. rainfall-evaporation cycle). The schematic diagram for the experimental procedure is shown in SI Figure S1(b).

**Text S6. Effect of SMX products.**

To investigate the effect of SMX transformation products, 100 μM SMX and 50 g/L MnO$_2$ was reacted in evaporation process for 6 days to obtain high concentration of SMX products. The degradation of SMX after 6 days had ceased, and then methanol was used as extracting solution to eliminate the effect of Mn$^{2+}$ due to the low solubility of Mn$^{2+}$ in methanol. SMX product and remaining SMX was extracted into methanol and re-diluted into H$_2$O after nitrogen blowing to replace methanol. The
ultimate mixture was three times diluted with the final SMX concentration was 1.85 μM. We assumed that SMX generated SMX products at 1:1, then the ultimate mixture was considered consisting of 1.85 μM SMX and 31.48 μM SMX products.

Another evaporation system was initially equipped with 20 μM SMX and 10 g/L MnO₂, which also reacted for 6 days to acquire the reaction plateau. The effect of SMX products was studied by adding the above mentioned mixture into this plateau system (SI Figure S9 (c)). The effect of H₂O and 20 μM SMX were also studied as contrast (SI Figure S9 (a) and (b)).
Figure S1. Schematic diagram for the experimental procedure
Figure S2. XRD pattern of selected MnO$_2$
Figure S3. SEM images of selected MnO$_2$
Figure S4. Degradation of SMX by MnO₂ in suspension systems at fixed SMX loading (20 μM) with varying MnO₂ loading from 10-100 g/L at pH 6.3.
Figure S5. (a) Degradation of SMX, and (b) Observed degradation rate constant ($k_{obs}$) of SMX, in suspension systems at fixed MnO$_2$ loading (10 g/L) with varying SMX loading from 20-200 μM at pH 6.3.
Figure S6. SMX degradation by MnO₂ in suspension system at pH 6.3 maintained with different concentrations of acetate buffer. Initial concentration of SMX was 20 μM, initial concentration of MnO₂ was 10 g/L. Reaction time was 6 days.
Figure S7. Degradation of SMX by MnO$_2$ in suspension. Initial concentration of SMX was 100 μM, initial concentration of MnO$_2$ was 10 g/L, pH was 6.3.
Figure S8. Degradation of SMX by fresh MnO$_2$ versus MnO$_2$ evaporated in buffer for 3 days. Initial concentration of SMX was 20 μM, initial concentration of MnO$_2$ was 10 g/L, pH was 6.3, reaction time was 3 days.
**Figure S9.** Adding 2mL of (a) H₂O, (b) 20 μM SMX, (c) mixture of 1.85 μM SMX and high concentration of SMX products, to the sample at the end of 6-day evaporation process. The additional reaction time was 3 days. (The experimental details are described in SI Text S6.)
Figure S10. Mn 2p XPS spectra of fresh $\gamma$-MnO$_2$ and reacted $\gamma$-MnO$_2$. The reacted MnO$_2$ was prepared after reacting with SMX in evaporation for several days. Initial concentration of SMX was 100 $\mu$M, initial concentration of MnO$_2$ was 10 g/L.
Figure S11. Mn 3s XPS spectra of (a) fresh $\gamma$-MnO$_2$; (b) $\gamma$-MnO$_2$ after reacting with SMX; (c) $\gamma$-MnO$_2$ after reacting with buffer (without SMX); (d) $\gamma$-MnO$_2$ after reacting with Mn$^{2+}$ (without SMX). Initial concentration of SMX was 100 μM, initial concentration of MnO$_2$ was 10 g/L, initial concentration of Mn$^{2+}$ was 100 μM.
Figure S12. Mn$^{2+}$ detected by ICP-OES. Initial concentration of SMX was 100 μM, initial concentration of MnO$_2$ was 10 g/L, reaction time was 5 days, the Mn$^{2+}$ adsorbed on MnO$_2$ surface was desorbed with 2% HNO$_3$. 

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0 2 4 6 8 10 12 14
suspension evaporation

Mn$^{2+}$ (mM)
Figure S13. The effect of Mn$^{2+}$ on the degradation of SMX in evaporation. Initial concentration of SMX was 20 μM, initial concentration of MnO$_2$ was 10 g/L, reaction time was 6 days.
Figure S14. Effect of rainfall on the degradation of SMX by MnO$_2$. Reaction condition: Initial concentration of SMX was 20 μM, initial concentration of MnO$_2$ was 10 g/L. In continued evaporation process, H$_2$O was evaporated to minimum amount within 3 days and kept constant to the end of the experiment, while in evaporation-rainfall cycle process, replenished initial volume of H$_2$O as rainfall every 3 days and repeated evaporation process. SMX/SMX$_0$ refers to the degradation rate of the total SMX amount in reaction system.
Figure S15. UPLC-MS/MS spectra of (a) SMX, (b) IP 1, (c) IP 2, (d) FP 1, (e) FP 2, and (f) FP 3.
Figure S16. NSMX degradation by MnO$_2$ in suspension system and evaporation process. Initial concentration of NSMX was 20 μM, initial concentration of MnO$_2$ was 10 g/L, pH was 6.3.
Figure S17. A similar structure for FP2.
Table S1. Parameters fitting for volume change

<table>
<thead>
<tr>
<th>time interval (day)</th>
<th>a</th>
<th>b</th>
<th>( V_t ) (L)</th>
</tr>
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<tbody>
<tr>
<td>0-0.5</td>
<td>-0.0014</td>
<td>0.002</td>
<td>(-0.0014t+0.002)</td>
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<tr>
<td>0.5-1.0</td>
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<td>0.0019</td>
<td>(-0.0013t+0.0019)</td>
</tr>
<tr>
<td>1.0-1.5</td>
<td>-0.0011</td>
<td>0.0017</td>
<td>(-0.0011t+0.0017)</td>
</tr>
<tr>
<td>1.5-2.0</td>
<td>-0.0001</td>
<td>0.0003</td>
<td>(-0.0001t+0.0003)</td>
</tr>
<tr>
<td>2.0-12.0</td>
<td>-</td>
<td>-</td>
<td>0.002*H₂O content</td>
</tr>
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</table>

The \( V_t \) within 0-2 day were piecewise fitted using measured water content, \( V_t \) after 2 days was considered constant in each sampling interval.
<table>
<thead>
<tr>
<th>Product Name</th>
<th>m/z</th>
<th>Structure</th>
<th>Reference</th>
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<tr>
<td></td>
<td>525.06 (+Na)</td>
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<tr>
<td>FP3</td>
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<td></td>
<td>370.08 (+Na)</td>
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**Table S3.** Molecular structure and molecular weight of SMX and NSMX.

<table>
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<tr>
<th>SAs</th>
<th>Molecular structure</th>
<th>Molecular weight</th>
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<tr>
<td>NSMX</td>
<td><img src="image" alt="NSMX Diagram" /></td>
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References


