The Acute Toxicity Change in the Microwave-enhanced Photolysis Degradation of Tetracycline

Wenchao Liao¹²*, Su Xu¹, Wenwen Chen¹ and Shuyi Liao²
¹ School of Environmental Science and Engineering, Xiamen University of Technology, Xiamen, Fujian 361024, China
² College of Health and Environmental Engineering, Shenzhen Technology University, Shenzhen, Guangdong 518118, China
*Corresponding author e-mail address: liaowenchao@sztu.edu.cn

Abstract. Antibiotic wastewater may generate more toxic by-products in the degradation process. It is of great significance to study the toxicity changes of the antibiotic intermediates for ecological safety of water environment. The microwave-enhanced photolysis process was set up and applied to the antibiotic tetracycline degradation. The luminescence inhibition rate of the luminescent bacteria was used as the evaluation index to investigate the acute toxicity changes of tetracycline intermediates. The toxicity changes with the degradation time and the effects of common water quality factors such as NO₃⁻, Cu²⁺ and humic acid on the acute toxicity of intermediates were studied. The results showed that nine intermediate products formed in the microwave-enhanced photolysis process. The acute toxicity of intermediates was generally reduced compared with tetracycline itself, but the luminescence inhibition rate of intermediates fluctuated with time. The luminescence inhibition rate of intermediates after 4 min of reaction was the highest, which meant the acute toxicity of intermediate products at 4 min was the largest. The presence of NO₃⁻ (high concentration) and Cu²⁺ increased the acute toxicity of tetracycline intermediates, but the presence of humic acid could help reduce the acute toxicity of tetracycline degradation intermediates. This study provided theoretical basis and data reference for the practical application and ecological risk assessment of microwave-enhanced photolysis degradation of tetracycline.

1. Introduction
Tetracyclines (TCs), which are widely used in clinical practice, are broad-spectrum antibiotics with significant inhibitory effect on most Gram-positive bacteria (G⁺) and Gram-negative bacteria (G⁻), Rickettsia, Chlamydia, Mycoplasma, Eperythrozoon, Borrelia, Actinomycetes, and some protozoa. According to the related research, a considerable part of tetracyclines entered the environment with body excreta in the form of prototype or metabolite. Due to its unbiodegradability, tetracycline is easily accumulated in water environment. Tetracycline has been detected in surface water, groundwater and the inlets and outlets of sewage treatment plants [1]. As environmental exogenous chemical, the discharged tetracycline had profound impact on environment and ecology, and eventually, it might cause irreversible serious impacts on human health and survival along the food chain [2,3]. The in-depth study of the toxicity changes during the degradation of tetracycline and the factors affecting the toxicity of tetracycline intermediates are of great significance to the ecological security of water environment.
The unbiodegradability of tetracycline makes it difficult to degrade by conventional water treatment techniques [4]. In recent years, the rapid development of advanced oxidation technologies such as photocatalysis technology, especially the development of combined photocatalysis technology with microwave or ultrasound, has effectively solved the bottleneck problem of traditional technology and achieved high antibiotic removals [5,6]. The microwave-enhanced photolysis process (MW/UV) was developed for the degradation of tetracycline. Since many intermediate products were generated during the degradation of tetracycline, and the biological toxicity of these intermediates was unknown, the ecological safety in the MW/UV degradation of tetracycline was uncertain. Therefore, the luminescent bacteria method was used to study the acute toxicity of tetracycline degradation intermediates formed in the MW/UV process, and the effects of common water quality factors on the acute toxicity of tetracycline degradation intermediates were investigated. This study would provide data support and theoretical reference to the application of the MW/UV technology, as well as the environmental behaviour, toxicological, ecological health and safety evaluation of tetracyclines.

2. Materials and Methods

2.1. Materials
Tetracycline, humic acid were both purchased from Aladdin Reagent Co., Ltd. All the chemicals were of analytical grade and used without further purification. BioFix® Lumi Multi-Shot luminescent bacteria, BioFix® Lumi activation solution and BioFix® Lumi control solutions were provided by Germany Macherey-Nagel company.

2.2. Experimental methods

2.2.1. Experimental procedure of the MW/UV degradation of tetracycline. In a typical MW/UV degradation of tetracycline, a glass reactor equipped with two U-type microwave discharged electrodeless UV lamp (MDEL, Shanghai Jiguang Special Illumination Instruments Factory) and 1 L tetracycline solution with the concentration of 500 μg/L was placed in an APEX microwave reaction system (Preekem Scientific Instruments Co., Ltd). The tetracycline solution was circulated in the reactor by peristaltic pump. Set the microwave power to 475W, and the related MDEL light intensity was 31.14 mW/cm² with the main emission wavelength at 254nm. Turned on the microwave, lighted the MDEL and started timing. Took samples after different reaction time intervals to test the acute toxicity of the reaction solution.

2.2.2. Acute toxicity test method. The luminescence inhibition rate of the BioFix® Lumi Multi-Shot luminescent bacteria was used as the evaluation index, and the BioFix® Lumi-10 portable water toxicity analyzer (Germany Macherey-Nagel company) was applied to test the acute toxicity of water samples of tetracycline. The specific test methods were as follows.

First adjusted the pH of the sample solution to maintain the pH between 6-8, then added 0.2 g of NaCl to each 10 mL of the sample solution to salinize the sample. At the same time, 6 mL of activation solution was added to the lyophilized bacteria to activate the luminescent bacteria. The activated luminescent bacteria were returned to the refrigerator and cultured at 2 °C-8 °C for 5 min for use. Injected 0.5 mL of the activated luminescent bacteria solution into the control test tube and the sample test tube, and left them at 15 °C for 10 min. After that, used water quality toxicity analyzer to test the background luminescence of the sample. Then added 500 μL of the control solution to the control tube and added 500 μL of the sample solution to the sample tube. Left them cultivated for 30min, and the luminous intensity of the luminescent bacteria was measured afterwards. Results were shown as Luminescence Inhibition Rate, which was calculated by the following formula.

\[
\text{Luminescence Inhibition Rate (%) = 1- (luminous intensity of the sample / luminous intensity of the control) \times 100%}
\]  

(1)
2.2.3. Tetracycline degradation intermediates analysis method. Tetracycline intermediates were detected by an ultra-high pressure liquid chromatography coupled with a quadrupole and a time-of-flight mass spectrometer (HPLC1290-Q-TOF, Agilent Corporation). The column was Zorbax Eclipse Plus C18 (2.1×50 mm, 1.8 Micron). The mobile phase was acetonitrile and methanol (60/40, v/v). The flow rate was 0.4 mL/min. The detection wavelength was 356 nm. The injection volume was 5 μL. The mass spectrometry test used an electrospray ionization mass spectrometry (ESI-MS) with positive ion mode (M+Na) +. The full scan acquisition ranged from 100 to 1100, and the spray voltage was 4000 V.

3. Results and discussion

3.1. Identification of tetracycline intermediates in the MW/UV process

Tetracycline intermediates formed after the MW/UV degradation of 0 min, 2 min and 4 min were analysed by HPLC-Q-TOF, respectively. According to the results of mass spectrometry analysis, nine main intermediate products with the m/z values of 465, 437, 430, 396, 305, 289, 278, 202 and 176 were detected. The molecular structures were analysed based on the m/z of intermediate products and the related references [7,8]. According to the proposed molecular structures, a large number of -OH formed in the MW/UV degradation process, the polycyclic structure of the parent tetracycline might be attacked by -OH to generate -OH substitution or removal products.

3.2. Changes in acute toxicity of the MW/UV degradation intermediates of tetracycline

Based on the analysis of intermediate products, the acute toxicity changes of the reaction solution were further investigated. Samples were taken at 1min, 3min, 4min, 6min and 9min to test the acute toxicity. The changes in the luminescence inhibition rate of the luminescent bacteria were shown in Figure 1.

![Figure 1. The luminescence inhibition rate trend of the MW/UV degradation intermediates of tetracycline.](image1)

Results showed that the luminescence inhibition rate of tetracycline intermediates fluctuated with the extension of reaction time. At 4 min, the luminescence inhibition rate reached the maximum, even higher than the tetracycline itself. The greater the luminescence inhibition rate, the higher the acute toxicity of the reaction solution. So, the intermediates produced at 4 min had the highest acute toxicity in this case. According to the mass spectrometry results of the intermediates, the products (m/z=305, 289, 278, 202 and 176) were generated after 4 min of the MW/UV reaction. Meantime, the simulation calculation by the EcoSAR model suggested that the acute toxicity of the intermediate products (m/z=305, 289, 278 and 202) on fish and daphnia were all greater than that of tetracycline itself. It
might be because the formation of more poisonous intermediates elevated the luminescence inhibition rate of the reaction solution withdrawn at 4 min. On the other hand, although the acute toxicity of intermediates fluctuated up and down within the reaction time of 0-9 min, the toxicity of intermediates in other reaction stages except for 4 min was generally less than that of tetracycline itself. The results indicated that the overall ecological and environmental risks reduced in the MW/UV degradation of tetracycline, and the MW/UV process was relatively safe for the ecological environment.

3.3. Effects of common water quality factors on the acute toxicity of the tetracycline intermediates in the MW/UV process

Anions, cations, and natural organic substances are common in water environment. The effects of the common ions NO₃⁻ and Cu²⁺, as well as the natural organic compounds humic acid on the acute toxicity of the tetracycline intermediates in the MW/UV process were investigated, and the results were shown in Figures 2, figure 3, and figure 4, respectively.

As can be seen from Figure 2, NO₃⁻ concentration significantly influenced the acute toxicity of the MW/UV intermediates of tetracycline. With the increase of the NO₃⁻ concentration, the luminescence inhibition rate gradually increased. When the NO₃⁻ concentration was low, the luminescence inhibition rate of the solution was smaller than the tetracycline raw water. However, when the NO₃⁻ concentration increased to 15 mg/L and 20 mg/L, the luminescence inhibition rate of the reaction solution exceeded that of the control solution without NO₃⁻. The luminescence inhibition rate was as high as 77% when the NO₃⁻ concentration was 20 mg/L. High concentration NO₃⁻ could inhibited the growth of light-emitting bacteria, which caused the death of luminous bacteria, and thus the luminescence inhibition rate increased. Experiments suggested that when using the MW/UV technology for the degradation of tetracycline in practical water, attention needed to be paid to high concentration NO₃⁻, especially to prevent the possible ecological risks caused by high concentration of NO₃⁻.

As shown in Figure 3, due to the addition of Cu²⁺, the luminescence inhibition rates of the tetracycline intermediates were all higher than the control solution without Cu²⁺. With the increase of the Cu²⁺ concentration, the luminescence inhibition rate first increased and then decreased. When the Cu²⁺ concentration increased to 1000 μg/L, the luminescence inhibition rate reached the maximum, which suggested the acute toxicity of the reaction solution was the highest.

**Figure 3.** Effect of Cu²⁺ on the luminescence inhibition rate of tetracycline degradation intermediates.

**Figure 4.** Effect of HA on the luminescence inhibition rate of tetracycline degradation intermediates.
According to the relevant reports, when adding different concentrations of Cu$^{2+}$, the absorption peak of tetracycline had a gradually red shift [9]. Changes in the absorption peak indicated that a new substance formed between tetracycline and Cu$^{2+}$. Referring to the acute toxicity results in this experiment, more toxic intermediates might be generated in the presence of Cu$^{2+}$. When applying the MW/UV process for the removal of tetracycline in practical water, attention needed to be paid to Cu$^{2+}$ to avoid the possible ecological and environmental risks.

Figure 4 suggested that the presence of humic acid was beneficial to reducing the luminescence inhibition rate of the luminescent bacteria, that was, humic acid was good to cut down the acute toxicity of the intermediate products. With the increase of humic acid concentration, the luminescence inhibition rate of the tetracycline intermediates first increased and then decreased. When the humic acid concentration was 20 mg/L, the luminescence inhibition rate was 9%, which was still obviously lower than that of the control solution without humic acid.

Studies have shown that new complexes might form by connecting humic acid with tetracycline via hydrogen-bond and π-π Bond, and the production of new complexes might inhibit the degradation of tetracycline [10]. Considering the acute toxicity results in this experiment, it was possible that humic acid and tetracycline produced new intermediates with lower acute toxicity in the MW/UV process. The presence of humic acid would not provide negative impact on the MW/UV degradation of tetracycline from the angle of ecological risk.

4. Conclusion
In the MW/UV process, tetracycline was degraded along the -OH substitution or removal pathways, and a total of 9 intermediate products yielded. The acute toxicity of tetracycline degradation intermediates was generally lower than that of tetracycline itself, but the luminescence inhibition rate of the intermediates generated after 4min of reaction was higher than that of the tetracycline raw water, indicating the formation of intermediates with higher acute toxicity at 4 min. The presence of common water quality factors NO$_3^-$ (high concentration) and Cu$^{2+}$ increased the luminescence inhibition rate and the acute toxicity of the tetracycline intermediates. Especially, when the NO$_3^-$ concentration increased to 20 mg/L, the luminescence inhibition rate reached 77%, which was significantly higher than the acute toxicity of tetracycline itself. It was necessary to be vigilant of the ecological risks caused by NO$_3^-$ (high concentration) and Cu$^{2+}$, when treating the tetracycline in practical water environment. In contrast, the presence of humic acid was generally beneficial to reducing the luminescence inhibition rate and the acute toxicity of tetracycline degradation intermediates. The results of these experiments could provide theoretical basis and data reference for the practical application and ecological risk assessment of the MW/UV degradation of tetracycline.

Acknowledgements
The authors are grateful for the financial support from the Natural Science Foundation of Fujian Province, China (No. 2018J01526) and the National Natural Science Foundation of China (No. 51408518).

References
[1] Xu K, Wang J, Gong H, Li Y, Zhou L, Yan M 2019 Ecotoxicology and Environmental Safety 186 109796
[2] Long S, Yang Y, Pavlostathis SG, Xiang F, Sun P, Li N, Zhao L 2020 Chemosphere 246 125681
[3] Chen CQ, Zheng L, Zhou JL, Zhao H 2017 Science of the Total Environment 580 1175-1184
[4] Cetecioglu Z, Incé B, Gros M, Rodríguez-Mozaz S, Barceló D, Orhön D, Incé O 2013 Water Research 47 2959-2969
[5] Wang W, Han Q, Zhu ZJ, Zhang LS, Zhong S, Liu BJ 2019 Advanced Powder Technology 30 1882-1896
[6] Adhikari S, Lee HH, Kim DH 2019 Chemical Engineering Journal doi.org/10.1016/j.cej.2019.123504
[7] Yuan F, Hu C, Hu X, Wei D, Chen Y, Qu J 2011 *Journal of Hazardous Materials* **185**(2-3) 1256-1263
[8] Khan MH, Bae H, Jung JY 2010 *Journal of Hazardous Materials* **181** 659-665
[9] Wen MQ, Gao YT, Luo YG, Wang HH 2005 *Photographic Science and Photochemistry* **23**(1) 71-78
[10] Liu F, Liu X, Zhao S, Wang J, Qian X, Cui B, Bai J 2019 *Chemosphere* **235** 867-875