Degradation and changes in toxicity and biodegradability of tetracycline during ozone/ultraviolet-based advanced oxidation

Huyen Trang Luu and Kisay Lee

ABSTRACT

Advanced oxidation processes (AOPs) composed of O_3, H_2O_2 and ultraviolet (UV) were applied to degrade tetracycline (TC). Degradation efficiency was evaluated in terms of changes in absorbance (ABS) and total organic carbon (TOC). The change in biotoxicity was monitored with *Escherichia coli* and *Vibrio fischeri*. The improvement in biodegradability during oxidation was demonstrated through 5-day biochemical oxygen demand/chemical oxygen demand ratio and aerobic biological treatment. The combination of O_3/H_2O_2/UV and O_3/UV showed the best performance for the reductions in ABS and TOC. However, mineralization and detoxification were not perfect under the experimental conditions that were used in this study. Therefore, for the ultimate treatment of TC compounds, it is suggested that AOP treatment is followed by biological treatment, utilizing enhanced biodegradability. In this study, aerobic biological treatment by *Pseudomonas putida* was performed for O_3/UV-treated TC. It was confirmed that O_3/UV treatment improved TOC reduction and facilitated complete mineralization in biological treatment.

Key words | advanced oxidation, biodegradability, biological treatment, biotoxicity, tetracycline

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INTRODUCTION

The presence of antibiotic compounds in livestock runoff and in discharges of pharmaceutical manufacturers or hospitals can cause the occurrence of antibiotic-resistant microorganisms which threaten normal functions of the ecosystem and public health. Therefore, the proper treatment of antibiotic compounds in wastewater treatment systems has become the subject of growing concern and scientific interest.

However, many antibiotics are not metabolized completely in animals and humans, but also are not completely removed in conventional wastewater treatment processes. These compounds are hardly biodegradable in usual biological processes due to their antibacterial nature (Yang *et al.* 2005; Kim *et al.* 2007). Advanced oxidation processes (AOPs) characterized by the generation of radical species including hydroxyl radicals have proved to be effective in the removal of many antibiotic compounds. Among the available AOP techniques, ozone-based advanced oxidation is considered as a prospective candidate because ozone itself is a good oxidation agent and also the source of hydroxyl radicals when it is coupled with ultraviolet irradiation or hydrogen peroxide (Legrini *et al.* 1993; Tambosi *et al.* 2009).

Even though advanced oxidation processes including ozone or ultraviolet (UV) are a known alternative option for the degradation of antibiotics like tetracyclines (TCs) (Oller *et al.* 2011; Yuan *et al.* 2011), treating antibiotic-containing wastewater by AOP alone is costly compared to treatment by biological processes. One way of reducing the treatment cost is first treating the antibiotic-containing wastewater with an AOP to degrade partially and to increase biodegradability, and then return to a traditional biological process to achieve further treatment (Yahiat *et al.* 2012), or vice versa.

The family of TCs, including TC, chlortetracycline (CTC) and oxytetracycline (OTC), is a widely used class of antibiotic compounds used as human and veterinary medicines. The degradation of TCs by AOP has been studied intensively. Wu *et al.* (2010) investigated the ozonation of TC, where the enhancement of biodegradability and the reduction in bioluminescent toxicity and chemical oxygen demand (COD) were achieved. Wang *et al.* (2011, 2012)

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showed the synergistic effect of ultrasound irradiation during the ozonation of TC. They suggested possible oxidation pathways and resulting decomposition intermediates. Lopez-Penalver *et al.* (2010) and Gomez-Pacheco *et al.* (2011) applied O_3/H_2O_2 for TCs' degradation and achieved an enhancement of biodegradability and toxicity reduction. They also carried out biological treatment with activated sludge after ozonation and confirmed an improved total organic carbon (TOC) reduction.

However, little information is available on the simultaneous use of UV with ozone and of the comparison of possible combinations of $O_3/UV/H_2O_2$. Also, the subsequent application of O_3/UV-treated TC to biological treatment has been limited. The objective of the present study was to evaluate the capability of O_3 or UV-based AOP to enhance the biodegradability of TC and the efficiency of subsequent biological treatment. The effectiveness of various AOP combinations using O_3, UV, and H_2O_2 was assessed for the mineralization and detoxification of TC. The changes in biotoxicity during AOP treatment were evaluated through the *Escherichia coli* susceptibility test and *Vibrio fischeri* bioluminescence test. The enhancement of biodegradability was assessed in terms of a 5-day biochemical oxygen demand (BOD_5)/COD ratio and, in order to confirm the possible merits of enhanced biodegradability, an aerobic biological treatment of AOP-treated TC was carried out using *Pseudomonas putida*, which is one of the major aerobic heterotrophic bacteria in the activated sludge process.

MATERIALS AND METHODS

Reagents and microorganisms

TC was purchased from Sigma-Aldrich (T7660). *E. coli* (ATCC 25922) and *P. putida* (ATCC 17514) were grown in LB-broth (25 g/L) at 37 °C and in soybean-casein digest (30 g/L) at 35 °C, respectively. *V. fischeri* (NRRL-B-11177), the marine photobacterium, was cultivated at 25 °C in a medium containing 10 g/L tryptone, 5 g/L yeast extract, and 25 g/L NaCl.

AOP treatment

The AOP reactor system used in this study has been described previously (Lee *et al.* 2011c). A closed, cylindrical acrylic reactor with a total volume of 1.2 L (height, 43 cm; diameter, 6 cm) was used. Ozone was produced by an ozone generator (LAB2B, Degremont Technologies, France) and injected into the lower part of the reactor to make 1.5 mg/L of dissolved ozone concentration. A 15 W low pressure mercury ultraviolet lamp was vertically installed inside the cylindrical AOP reactor with 2 cm of averaged irradiation distance. The UV lamp has dimensions of 25.5 × 436 mm and 4.9 W UV output, which irradiates UV light of 254 nm. The temperature of the reactor was maintained at 20 °C by circulating water through the outside jacket using a water circulator. AOP experiments were conducted with initial TC concentration of 20 mg/L in deionized distilled water. One litre of TC solution was treated by different AOP combinations. Hydrogen peroxide concentration was fixed at 100 mg/L. Immediately after withdrawing at a designated time interval, the samples were flushed with nitrogen for 3 min at 15 mL/min in order to remove residual ozone before analysis.

Biotoxicity tests

Antibiotic susceptibility tests were carried out with *E. coli* using the Kirby-Bauer disk diffusion method (Bauer *et al.* 1966), in which the zone of inhibition was formed around the colony if antibiotic toxicity existed. The toxicity of parent compounds and their oxidation byproducts were also estimated by a biofluorescence assay (Froehner *et al.* 2000; Jennings *et al.* 2001) with *V. fischeri* using a TD-20/20 Luminometer (Turner Designs, USA). The fluorescent light emitted from *V. fischeri* is the result of interaction of the luciferase enzyme (Hernando *et al.* 2007). The inhibition of the biofluorescence of *V. fischeri* was utilized as an indication of acute toxicity.

Biological treatment

An O_3/UV-treated TC solution was added to artificial wastewater and treated by aerobic biological process using *P. putida*, which is one of the major aerobic heterotrophic bacteria in the activated sludge process. The detailed experimental setup and conditions have been described previously (Luu 2013). Biological treatment was performed in acrylic rectangular reactors with a square bottom (15 cm × 15 cm) and 5-L working volume. Agitation was achieved by aeration through the bottom diffuser at the rate of 0.03 vvm. Batch treatment experiments were carried out with three reactors in parallel, which consisted of (i) control (no TC added), (ii) untreated TC (with 20 mg/L of raw TC), and (iii) AOP-treated TC (with 20 mg/L TC which was treated by O_3/UV for 15 min). The initial concentration of

P. putida was fixed at around 14 mg/L. The artificial wastewater had the ratio of BOD_5:N:P = 100:20:1 and organic carbon was made from glucose as 350 mg/L. During the course of the experiments, pH was varied between 6 and 8 and dissolved oxygen was kept around 8 mg/L at 20 °C.

Analysis methods

A UV-Vis spectrophotometer (DR/4000 HACH) was used to analyze TC absorbance (ABS) in aqueous solution at 272 nm. TOC was analyzed with a V-TOC Analyzer (Shimadzu). Dissolved ozone concentration was determined by the Indigo method (APHA 2005). The change in biodegradability during AOP treatment was evaluated by the changes of the BOD_5/COD ratio. Since hydrogen peroxide is not only a potential biocide (Linley *et al*. 2012) but also interferes with COD analysis (Lee *et al*. 2011a), residual hydrogen peroxide was eliminated through the reaction with sodium carbonate after H_2O_2-involved AOP treatments (Wu & Englehardt 2012). Residual ozone was also removed by purging the solution with nitrogen gas before analyzing BOD. All the experiments and analyses were performed more than three times and the data were averaged.

RESULTS AND DISCUSSION

Degradation of TC

The degradation of TC by various AOP combinations was investigated and the changes in ABS and TOC are shown in Figure 1. All combinations showed substantial ABS reduction except H_2O_2 or UV alone; the ABS of the parent compounds was almost completely removed within 30 min. The combination of O_3/H_2O_2/UV achieved the highest ABS reduction, and O_3/UV was the second. It is noted that O_3 alone was pretty effective in ABS reduction. The ABS reduction can occur from partial changes or modification of light-absorbing functional groups in the TC structure, and thus the ABS reduction is not considered as evidence of complete degradation nor mineralization. Usually oxidative degradation of simple organic pollutants can lead to the decomposition of structure and eventually to mineralization. However, the complete mineralization of most antibiotics is often difficult due to their structural complexity and stability (Paulou & Langlais 1999; Lee *et al*. 2011b).

In Figure 1, TOC reduction was 77% by O_3/H_2O_2/UV, 70% by the O_3/UV process, and around 50% by H_2O_2/UV, within 1 h of operation. It was also confirmed that TOC reduction by O_3 alone was much less than those by other AOP combinations, although its ABS reduction was significant. Dalmázio *et al*. (2007) indicated that the extent of TC mineralization was low in spite of rapid disappearance of the parent TC molecules by ozonation. The low degree of mineralization is probably due to the formation of stable intermediate products.

Changes in biotoxicity

The occurrence of partially oxidized (or partially degraded) intermediates during AOP treatment can influence toxicity and biodegradability. Figure 2 shows the changes in *E. coli* susceptibility and *V. fischeri* biofluorescence. The existence of H_2O_2 interferes with the correct

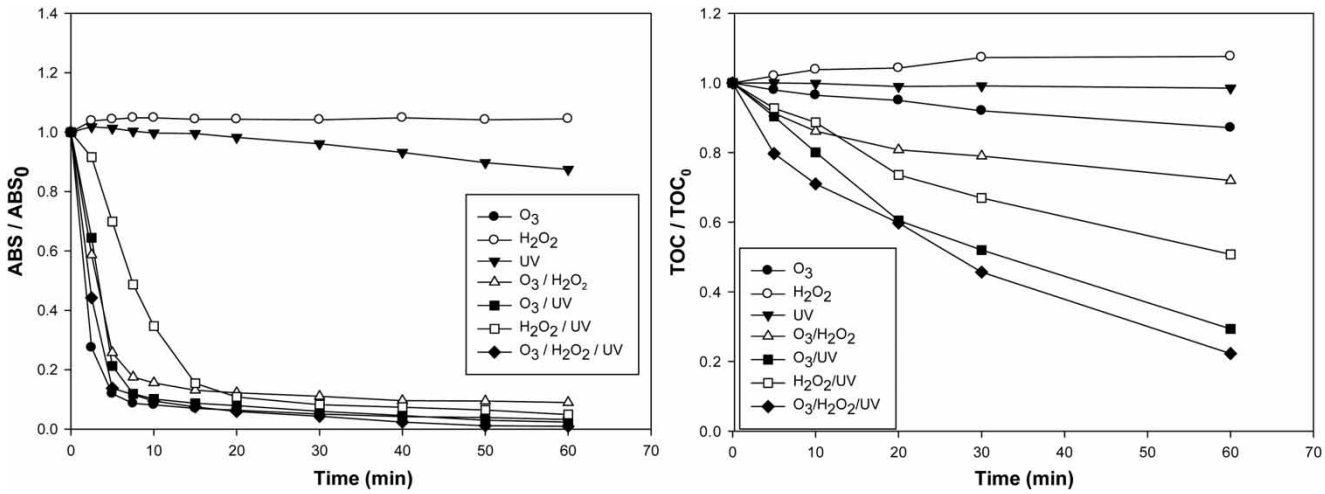

Figure 1 | Changes in ABS and TOC value during advanced oxidation of tetracycline.

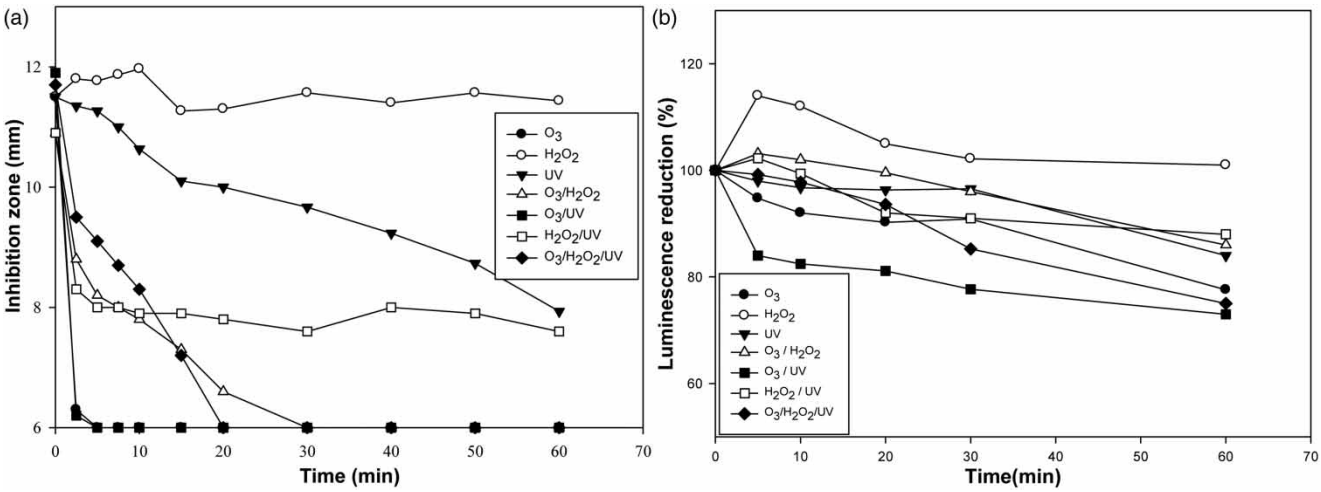

Figure 2 | Changes in (a) *E. coli* susceptibility and (b) *V. fischeri* bioluminescence during advanced oxidation of tetracycline.

assessment of biotoxicity and the performance of biological treatments due to its biocidal effect (Linley *et al.* 2012). Also its oxidation potential interferes with COD analysis because residual H_2O_2 consumes the employed oxidation agents (Lee *et al.* 2011a). Therefore, residual hydrogen peroxide was eliminated through reaction with sodium carbonate (Wu & Englehardt 2012) prior to biotoxicity tests and biological treatments.

Figure 2(a) shows that the diameter of the inhibition zone was decreasing quickly by ozone alone and by O_3/UV treatment, down to below the detection limit after 5 min of treatment. O_3/H_2O_2/UV and O_3/H_2O_2 also showed a substantial reduction of luminescence inhibition within 30 min. It was noted that the degradation products, especially in H_2O_2 treatment, may still possess antibacterial activity and thus bacteria could be more susceptible to them than to parent compounds (Witte *et al.* 2010).

Figure 2(b) shows the changes in biofluorescence of *V. fischeri* during various AOP treatments. The degree of inhibition of the untreated TC was considered to be 100%. Overall, O_3/UV and O_3/H_2O_2/UV exhibited the best performance in toxicity reduction. However, toxicity reduction in terms of *V. fischeri* biofluorescence was only up to 27% for 60 min, which indicated that the antibiotic toxicity to *V. fischeri* biofluorescence lasted longer than *E. coli* susceptibility.

Toxicity can sometimes even increase temporarily in the middle of AOP treatments. In the cases of H_2O_2, O_3/H_2O_2, and H_2O_2/UV, toxicity started increasing from the beginning and over 100% inhibition was maintained, implying that the involvement of H_2O_2 may produce toxic and stable intermediates against *V. fischeri* fluorescence. It was confirmed that an inappropriate AOP treatment can make toxicity worse than the original compounds (Li *et al.* 2008; Yuan *et al.* 2011).

Changes in biodegradability during the O_3/UV process

From the results in Figures 1 and 2, the treatments of O_3/H_2O_2/UV and O_3/UV showed best performance in TOC reduction and toxicity mitigation of a TC solution. Since their performances were not significantly different, the O_3/UV treatment was chosen for an optimal combination for TC treatment in this study.

Figure 3 shows the change of BOD_5/COD ratio, which is an index of biodegradability, during O_3/UV treatment. It

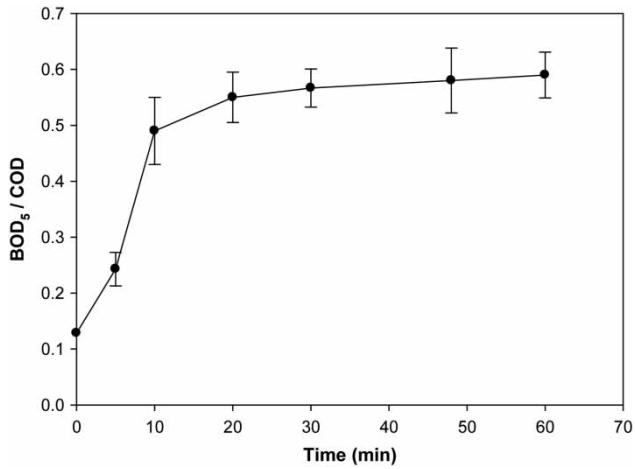

Figure 3 | The change in BOD_5/COD ratio during O_3/UV treatment of tetracycline. Error bars represent standard deviation for three replicates.

can be seen that the biodegradability of TC was improved successfully by O_3/UV application. The increase of the BOD_5/COD ratio was more than four-fold up to 30 min of treatment. The increase in BOD_5/COD ratio indicates that the refractory TC became biodegradable by the O_3/UV process. Previous studies have reported that TC and CTC became biodegradable after 10 minutes of AOP, while OTC took a longer time due to slight differences in the parent structure (Alaton & Caglayan 2006; Jeong *et al*. 2010). The results of Figure 3 implied that TC molecules which were treated by O_3/UV could become sufficiently biodegradable and ready for biological treatment processes.

Biological treatment with *P. putida* for O_3/UV-treated tetracycline

The merit of the enhanced biodegradability (Figure 3), achieved by O_3/UV application, was proved through actual biological treatment using *P. putida* (Figure 4). The control reactor contained wastewater only with 150 ppm of glucose TOC, without TC. The raw sample contained 20 mg/L of untreated raw TC and the treated sample contained 20 mg/L of TC which was treated by O_3/UV for 15 min.

In Figure 4, TOC values showed an acclimating period at the beginning in the biological reactor and then decreased rapidly within 1–2 days. The reductions in TOC are the results from the assimilation of glucose in artificial wastewater and tetracycline molecules. Because the concentration of TC was extremely small compared to other carbon constituents in wastewater, the actual disappearance of TC could not be identified by high-performance liquid chromotography during the treatment period. However, possible benefits of O_3/UV treatment of TC before biological treatment can be reflected in the differences of the reduction rates of TOC values. At the beginning of biological treatment, there existed a slight increase of TOC values, which appeared to be due to more complex compounds being generated temporarily from the reaction between AOP-treated intermediates and microorganisms (Alaton *et al*. 2004; Oller *et al*. 2011).

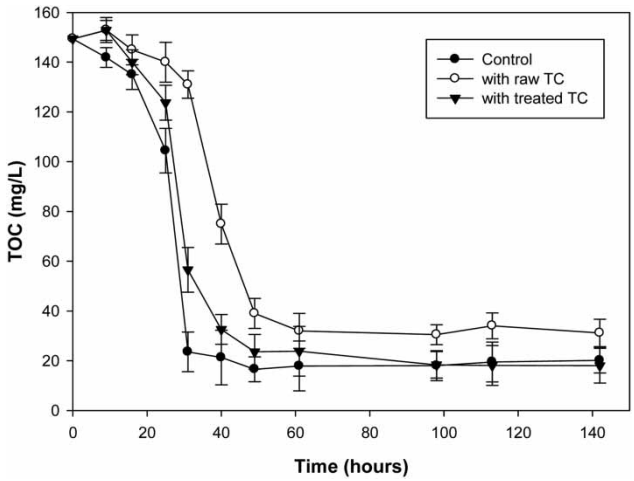

Figure 4 | TOC reduction by aerobic biological treatment of tetracycline-containing wastewater. Error bars represent standard deviation for three replicates.

The TOC values of O_3/UV-treated samples were always lower than those of raw samples (that is, untreated samples) throughout the course of biological treatment, indicating that the biological removal of treated samples proceeded faster. In other words, treated samples were less toxic to bacteria than raw samples. After 100 h of biological treatment, the TOC value of treated samples became comparable with that of the control reactor.

It can be concluded that O_3/UV pretreatment was beneficial for the aerobic biological process to accelerate the removal of TOC value compared with the direct treatment of raw TC, because O_3/UV treatment allowed the increase of biodegradability of TC molecules and the reduction of potential toxicity to the microorganisms in the bioreactor, as shown in Figures 3 and 4.

CONCLUSIONS

The degradation of tetracycline was investigated by various combinations of O_3, H_2O_2 and UV. By considering the changes in ABS, COD, TOC, toxicity and biodegradability, the O_3/UV combination appeared to be the best overall choice. Although ABS was reduced quickly for a given period of treatment, TOC results showed that the complete mineralization of the antibiotics was not achieved under AOP conditions used in this study. This is because the reduction of ABS reflects the change in light-absorbing characteristics of the molecular structure. However, the BOD_5/COD ratio was increased substantially and thus the O_3/UV combination was considered as a prospective choice for the pretreatment AOP before aerobic biological processes. O_3/UV pretreatment was indeed beneficial for the aerobic biological process using *P. putida* to accelerate TOC removal, taking advantage of the enhanced biodegradability of TC and the lowered toxicity to the microorganisms in the bioreactor.

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