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Article

고도산화공정 처리가 페니실린의 생독성, 생분해도 및 생물학적 분해에 미치는 영향

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Effects of Advanced Oxidation of Penicillin on Biotoxicity, Biodegradability and Subsequent Biological Treatment

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초 록

페니실린(PEN) 항생제의 분해를 위하여 오존, 과산화수소, 자외선으로 구성된 고도산화공정(AOP)을 적용하였다. 항생물질 분해효율은 흡광도(ABS) 및 총유기탄소(TOC) 분석으로 평가하였다. O_3/H_2O_2/UV와 O_3/UV 조합이 ABS (9 min 동안 100%) 및 TOC 감소(60 min 동안 70%)에 가장 효과가 좋았으나 사용한 실험조건에서 항생제의 무기질화 및 독성 제거는 완전하지 않았다. 항생물질에 의한 생독성은 *Escherichia coli* 민감도 및 *Vibrio fischeri* 생체형광 활성평가를 이용하였으며 O_3/UV에 의해 민감도는 9 min 동안 100% 감소, O_3/H_2O_2/UV에 의한 생체형광에 대한 독성은 60 min 동안 57% 감소하였다. 생물학적 분해를 위한 AOP 조합으로 O_3/UV 조합을 선정하였으며 BOD_5/COD 비율로 생분해도의 개선 여부를 간접 측정한 결과 O_3/UV로 30 min 처리함으로 BOD_5/COD 비율이 약 4배 증가하였다. 페니실린 20 mg/L를 포함하는 인공폐수에 대하여 AOP 처리 후 *Pseudomonas putida*를 이용하여 호기적 생물학적 분해를 진행한 결과, O_3/UV 전처리한 경우 페니실린의 완전 무기질화가 가능하였으며 전처리하지 않은 경우에 비하여 분해속도가 55% 증진되었다. 결론으로, 호기성 생물학적 처리를 위한 AOP 전처리로써 O_3/UV 조합이 추천되며 페니실린의 완전 분해를 촉진할 수 있다.

Abstract

Advanced oxidation processes (AOPs) composed of O_3 and UV were applied to degrade penicillin (PEN). The degradation efficiency was evaluated in terms of changes in the absorbance (ABS) and total organic carbon (TOC). The combination of O_3/H_2O_2/UV and O_3/UV showed the best performance for the reduction of ABS (100% for 9 min) and TOC (70% for 60 min) values, although the mineralization was uncompleted under the experimental condition in this study. The change in biotoxicy was monitored with *Escherichia coli* susceptibility and *Vibrio fischeri* biofluorescence. The *E. coli* susceptibility was eliminated completely for 9 min by O_3/UV, and the toxicity to *V. fischeri* biofluorescence was 57% reduced by O_3/H_2O_2/UV. For the ultimate treatment of PEN, it is suggested that an AOP using O_3/UV is followed by biological treatment, utilizing the enhanced biodegradability by the AOP. During 30 min of O_3/UV treatment, the BOD_5/COD ratio as an indication of biodegradability showed about 4-fold increment, compared to that of using a non-treated sample. TOC removal rate for AOP-pretreated PEN wastewater increased 55% compared to that of using the non-pretreated one through an aerobic biological treatment by *Pseudomonas putida* for artificial wastewater containing 20 mg/L of PEN. In conclusion, O_3/UV process is recommended as a pretreatment step prior to an aerobic biological process to improve the ultimate degradation of penicillin.

Keywords: *Advanced oxidation, biodegradability, biological treatment, biotoxicity, penicillin*

1. Introduction

The presence of antibiotic compounds in livestock runoff or in discharges from pharmaceutical manufacturers and hospitals can cause the occurrence of antibiotic-resistant microorganisms that threaten normal functions of ecosystem. The proper treatments of antibiotic compounds in wastewater treatment systems have become the subject of growing concern and scientific interests.

However, many of antibiotics are not metabolized completely in the body of human and animals, but also not completely removed in con-

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ventional wastewater treatment processes. These compounds are hardly biodegradable in usual biological processes due to their antibacterial nature[1,2]. In spite of anaerobic-aerobic combined biological treatment processes, the removal efficiency of antibiotic compounds are usually not satisfactory[3]. Physico-chemical methods such as direct and indirect photolysis and hydrolysis also showed a low efficiency of degradation[4].

Advanced oxidation processes (AOPs), which are characterized by the generation of radical species including hydroxyl radical, have proved to be effective in the removal of antibiotic compounds. Among the available AOP techniques, ozone-base advanced oxidation is considered as a prospective candidate because ozone itself is a good oxidation agent and also a source of hydroxyl radical when it is coupled with ultraviolet (UV) irradiation or hydrogen peroxide[5,6].

However, even though advanced oxidation processes (AOPs) including ozone or UV are an effective option for the degradation of antibiotics like penicillin[7,8], the treatment of antibiotics-containing wastewater by AOP alone is costly compared to the treatment by biological processes. One way to reduce the treatment cost is treating the antibiotic wastewater firstly by an AOP to degrade the targets partially and to enhance biodegradability, before sending it to biological process, or vice versa[9,10].

The objective of the present study was to evaluate the capability of O_3 or UV-based AOP to enhance biodegradability of penicillin (PEN) and the efficiency of biological treatment. Penicillin-family antibiotics (penicillin, ampicillin and amoxicillin) are the widely used class of antibiotic compounds as human and veterinary medicine. The effectiveness of the various AOP combinations using O_3, UV and H_2O_2 was assessed for the mineralization and detoxification of penicillin. The changes in biotoxicity during AOP treatment were evaluated though *Escherichia coli* susceptibility test and *Vibrio fischeri* biofluorescence test. The enhancement of biodegradability was assessed and, in order to confirm the possible merits of enhanced biodegradability, an aerobic biological treatment of AOP-treated PEN was carried out using *Pseudomonas putida*. The results and suggested strategy of this study are applicable to decide what kind of pretreatment process should be selected and how to evaluate its achievable potential advantages to improve overall removal efficiency of biological processes to treat antibiotics-containing wastewater.

2. Materials and Methods

2.1. Reagents and microorganisms

Penicillin G potassium salt was purchased from Sigma-Aldrich (P7794). *Escherichia coli* (ATCC 25922) and *Pseudomonas putida* (ATCC 17514) were grown in LB broth (25 g/L) at 37 ℃ and in soybean-casein digest (30 g/L) at 35 ℃, respectively. *Vibrio fischeri* (NRRL-B-11177), the marine photobacterium, was cultivated at 25 ℃ in the medium containing 10 g/L tryptone, 5 g/L yeast extract and 25 g/L NaCl.

2.2. AOP system

The AOP reactor system used in this study was described previously [11]. Ozone was produced by an ozone generator (LAB2B, Degremont Technologies, France) and injected to the reactor to make 1.5 mg/L of dissolved ozone concentration. A 15W low pressure mercury ultraviolet lamp (Kumho, Korea) was vertically installed inside the cylindrical AOP reactor with 2 cm of averaged irradiation distance. The lamp irradiates a UV light of 254 nm. The temperature of the reactor was maintained at 20 ℃ by circulating water using as water circulator through outside jacket. AOP experiments were conducted with initial PEN concentration of 20 mg/L in deionized distilled water. One liter of PEN-containing solution was treated by different AOP schemes. Hydrogen peroxide concentration was fixed at 100 mg/L. Immediately after withdrawing at designated time intervals, the samples were flushed with nitrogen for 3 min at 15 mL/min in order to remove residual ozone before analysis.

2.3. Biotoxicity tests

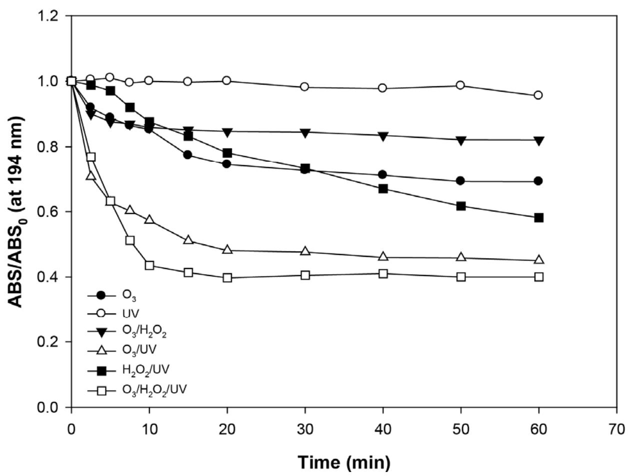
Antibiotic susceptibility tests were carried out with *E. coli* using the Kirby-Bauer disk diffusion method[12], in which the zone of inhibition was formed around the colony if the antibiotic toxicity existed. The toxicity of parent compounds and their oxidation by-products was also analyzed by the bioluminescence assay[13] with *V. fischeri* using TD-20/20 luminometer (Turner Designs, USA). The fluorescence light emitted from *V. fischeri* is the result of interaction of the luciferase enzyme[14] and the inhibition of the biofluorescence of *V. fischeri* is considered as an indication of acute toxicity.

2.4. Biological treatment

O_3/UV-treated PEN solution was added to artificial wastewater and treated by aerobic biological process using *P. putida*, which is a major culturable aerobic bacterium in activated sludge process. Biological treatment system is composed of three reactors in parallel; (i) control (no PEN addition), (ii) untreated sample (with 20 mg/L of raw PEN) and (iii) AOP-treated sample (with 20 mg/L PEN treated by O_3/UV for 15 min). The initial cell concentration of *P. putida* was fixed at around 14 mg/L. Artificial wastewater has the ratio of BOD_5 : N : P = 100 : 20 : 1 and organic carbon was made of glucose as 350 mg/L[15]. During the course of experiments, pH was varied between 6 and 8 and dissolved oxygen (DO) was kept around 8 mg/L at 20 ℃.

2.5. Analysis methods

A UV-vis spectrophotometer (DR/4000-U HACH) was used to analyze PEN absorbance in aqueous solution at 272 nm. Total organic carbon (TOC) was analyzed with V-TOC Analyzer (Shimadzu). Dissolved ozone concentration was determined by Indigo method[16]. The change in biodegradability due to AOP treatment was evaluated by the changes of the BOD_5/COD. Before analyzing BOD, residual ozone was removed by nitrogen gas purging.



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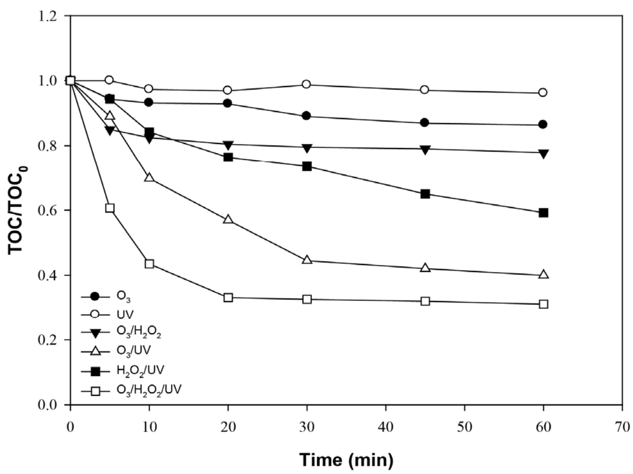
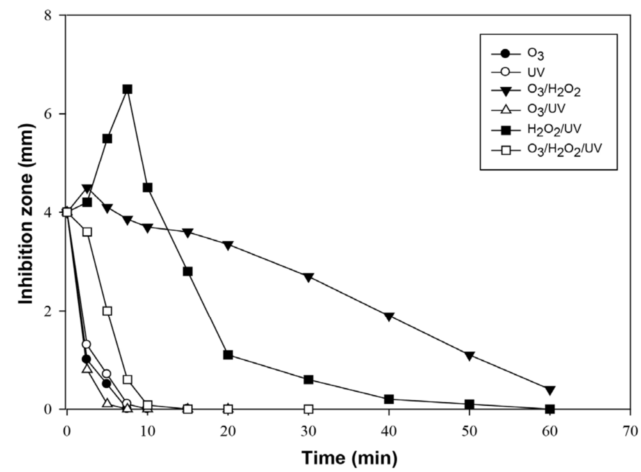


(b)

Figure 1. Changes in absorbance and TOC during advanced oxidation of penicillin.



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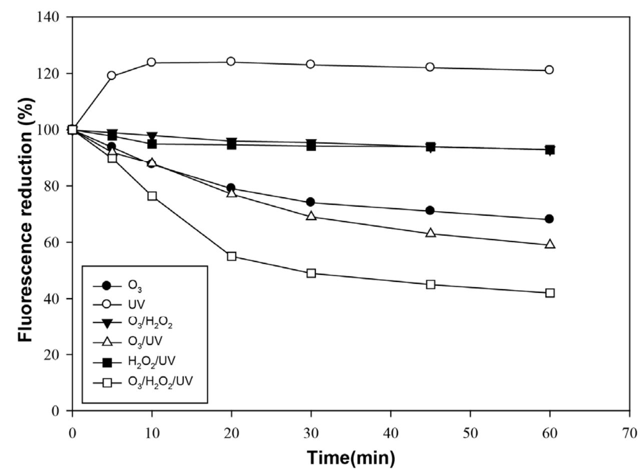

(b)

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

3. Results and Discussion

3.1. AOP treatments of penicillin

The degradation of penicillin by various AOP combinations was investigated and changes in absorbance (ABS) and TOC were shown in Figure 1. All combinations showed a substantial ABS reduction except H_2O_2 and UV alone; the almost of absorbance of parent compounds was removed within 30 min. The combination of $O_3/H_2O_2/UV$ achieved the highest ABS reduction and then O_3/UV was the second. It is noted that O_3 alone was very effective in absorbance reduction. The absorbance reduction can occur from the partial change or modification of light-absorbing functional groups in the PEN structure, and thus the absorbance reduction is not considered as an evidence of complete degradation nor mineralization. Although oxidative degradation of organic pollutants can lead to the decomposition of structure and eventually to the mineralization sometimes, the complete mineralization of most antibiotics is often difficult due to their structural stability[8,17].

In Figure 1, TOC reduction was 77% by $O_3/H_2O_2/UV$, 70% by O_3/UV process and around 50% by H_2O_2/UV, in 1 h 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.[18] also indicated that the extent of PEN mineralization was low in spite of quick disappearance of the parent PEN molecules and the low degree of mineralization is probably due to the formation of stable intermediate products.

3.2. Changes in biotoxicity

The 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. Figure 2a shows that the diameter of inhibition zone, which is the relative extent of susceptibility to toxicity, was decreasing quickly by ozone alone and by O_3/UV treatment, down to below 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 inhibition within 30 min. It was also observed that degradation products, especially in H_2O_2 treatment, may

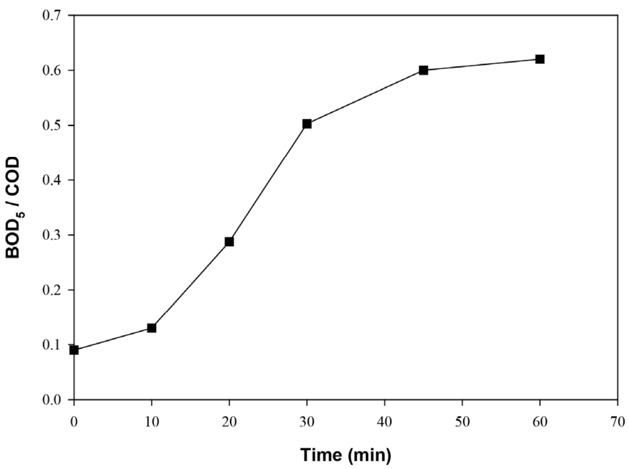

Figure 3. The change in BOD_5/COD ratio during O_3/UV treatment of penicillin.

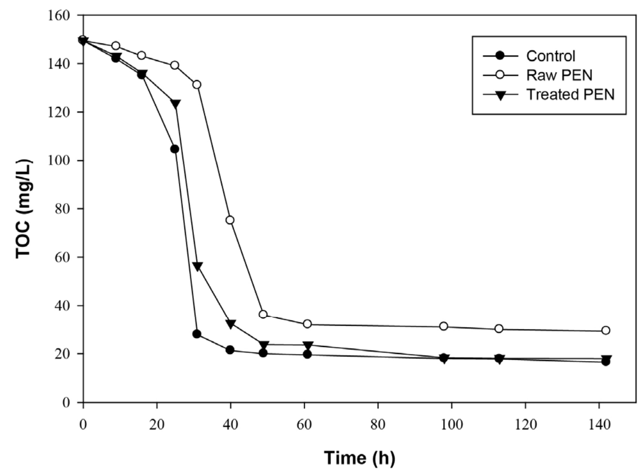

Figure 4. TOC reduction by aerobic biological treatment of penicillin-containing wastewater.

still possess antibacterial activity, even more susceptible with *E. coli* than parent compounds[19,20].

Figure 2b is the changes in biofluorescence of *V. fischeri* during various AOP treatments. The degree of inhibition by untreated PEN was considered 100%. O_3/UV and O_3/H_2O_2/UV exhibited the best performance overall in toxicity reduction. However, biotoxicity in terms of *V. fischeri* fluorescence reduction was 57% reduced for 60 min, which indicated that the antibiotic toxicity to *V. fischeri* fluorescence lasted longer than *E. coli* susceptibility.

Toxicity can even increase during AOP treatments sometimes. Especially, in the cases of O_3/H_2O_2 and H_2O_2/UV, *E. coli* susceptibility increased temporarily at early stage of AOP treatment and then decreased rapidly (Figure 2a). This is probably because the involvement of H_2O_2 oxidation produces more toxic intermediates than the parent PEN, but they are soon degraded further although it was not experimentally confirmed. Figure 2b also shows that *V. fischeri* bioluminescence increased by UV alone and maintained constantly, implying that stable intermediates against toxicity started increasing from the beginning and over 100% inhibition was maintained, implying that UV is responsible for generating stable intermediates or structural change which affects against *V. fischeri* luminescence. It was confirmed that an inappropriate AOP treatment can make toxicity worse than original compounds[7,21].

3.3. Changes in biodegradability during 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 the best performance in TOC reduction and toxicity mitigation of penicillin solution. Since their performances were not different significantly, the O_3/UV treatment was chosen for a selected combination for PEN treatment for later experiments.

Figure 3 shows the change of BOD_5/COD ratio, which is an index of biodegradability, during O_3/UV treatment for penicillin. It can be seen that the biodegradability of PEN was improved successfully by O_3/UV application. The increase of BOD_5/COD ratio was more than 4 folds until 30 min of the treatment. This increase in BOD_5/COD ratio indicates that the refractory PEN was changed more biodegradable by the O_3/UV process due to its characteristics of oxidation reaction. It has been reported that some antibiotics become biodegradable after AOP, and the extent of biodegradability change depends upon the structure of parent molecule such as functional group and bonding characteristics[22-24]. The results of Figure 3 implied that PEN molecules which were treated by O_3/UV could become sufficiently biodegradable and ready for biological treatment processes.

3.4. Biological treatment with *P. putida*

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

TOC values showed an acclimating period at the beginning in biological reactor and then decreased rapidly within 1-2 days. The reductions in TOC are the results from the assimilation of organic carbons such as glucose and penicillin. Because the concentration of PEN was extremely small among total carbon constituents in wastewater, the actual disappearance of PEN could not be identified by HPLC during treatment period. However, possible benefits of AOP treatment of PEN for biological treatment can be reflected in the differences of reduction rates of TOC values. By assuming the removal curves follow negative exponential decay before reaching stabilized phase (30-50 min) and comparing exponential coefficients, TOC removal rate in the raw PEN sample was 51.3% of that of control, while the removal rate in the treated PEN sample was up to 79.5% of control which was about 55% higher than that in raw PEN sample.

The TOC values of O_3/UV-treated sample were always lower than those of raw sample (that is, untreated sample), indicating that the biological removal of treated sample occurred faster. In other words, treated sample was less toxic to bacteria than raw sample. After 100 h of biological treatment, the TOC value of treated sample became comparable to that of the control reactor. Sometimes TOC value can increase slightly during the treatment in case that more complex compounds are generated from the reaction between AOP-treated intermediates and microorganism[9,25].

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

4. Conclusions

The degradation of penicillin was investigated by various combinations of O_3, H_2O_2 and UV. A complete degradation or mineralization was not guaranteed although ABS was reduced substantially by AOP because the reduction of ABS reflected the change in light-absorbing characteristics of the molecular structure of the compounds. TOC results showed that the complete mineralization of the antibiotics did not occur under AOP conditions used in this study. Although O_3/UV combination was best overall for ABS reduction, the TOC and toxicity reductions were not complete. However, the BOD_5/COD ratio was increased substantially and thus O_3/UV combination was considered as an optimal choice of AOP when it is combined with biological treatment. O_3/UV pretreatment was indeed beneficial for aerobic biological process using *P. putida* to accelerate TOC removal because O_3/UV treatment allowed the increase in the biodegradability of PEN and the reduction of potential toxicity to the microorganisms in bioreactor. In conclusion, O_3/UV process can be recommended as a pretreatment step prior to aerobic biological process to improve the ultimate degradation of penicillin.

Acknowledgements

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