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Understanding the algal contribution in combined UV-algae treatment to remove antibiotic cefradine

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Abstract

The aim of this study is to investigate the algal contribution in a combined UV-algae treatment to remove the commonly used antibiotic cefradine. We evaluated the removal capacity of the individual alga (*Chlorella pyrenoidosa*), UV and combined UV-algae treatment respectively. The toxic effect of the effluents treated by the above process on the standard test organism rotifer (*Brachionus calyciflorus*) was also investigated. Our results showed that the individual algae treatment was inefficient at removing the antibiotic. Although the UV treatment decreased the antibiotic concentration efficiently (26.93% residue), it also increased the toxicity of the effluent (1.04 times of the parent compound). However, the relatively high removal efficiency (22.01% residue) and the reduced toxicity of the effluent (nearly half of that by the individual UV treatment) were obtained synchronously after the UV-algae combined treatment. The contribution of the algae was also confirmed by the corresponding properties between the combined UV-activated sludge treatment and the combined UV-activated sludge-algae treatment. Our present study supported our hypothesis that (1) the performance of the individual algae was inferior to the individual UV irradiation; (2) the algae treatment was necessary in a UV-algae combined treatment system to control the toxicity of the effluent after the UV treatment process.

Keywords

Cefradine wastewater treatment, combined UV-Algal removal, algal contribution, toxicity control, rotifer assessment
Introduction

Antibiotics are commonly used in reducing the burden of common infectious diseases in human therapy and veterinary medicines as well as in promoting growth in livestock and aquaculture operations \(^1\). During the last few decades, the consumption of antibiotics has increased rapidly \(^2\), especially in China \(^3\). Antibiotics can be only partially metabolized by humans and animals and therefore the non-metabolized fractions are excreted into the effluents or transported to municipal wastewater treatment plants (WWTPs)\(^4\). Due to the low removal efficiency by the current treatment technology, WWTPs have become one of the dominant pollution sources for antibiotics\(^5\), the antibiotic residues have also been detected in the aquatic environment \(^6\). Considering their long-term use and bio-accumulation, the antibiotics may also endanger aquatic ecosystems \(^7\). Several studies have shown the toxic effects of the antibiotics on aquatic species \(^8,9\). In addition, presence and spread of antibiotics into the environment have also arisen antibiotic resistance in bacteria population, which would eventually affect human health \(^10\).

The common wastewater treatment technologies include bio-degradation, chemical degradation and physical degradation. Even though the conventional biological methods are the economical choice of treatment, several types of industrial wastewater, which from petrochemical, pharmaceutical or pesticide manufacturing plants, usually contain considerable amount of non-biodegradable organic compounds. Although the activated sludge is widely used in biological treatment process\(^11\), the above organic compounds are refractory to the microorganisms applied. For
antibiotics, the strong impact of the drugs on microorganisms usually leads to a low
treatment efficiency. However, the selective press also leads to several bacterial
strains easily acquire resistance against the antibiotics impact and release their
antibiotic resistance genes (ARGs) into the environment. Chemical treatments,
especially the advanced oxidation processes (AOPs), are influential treatment
methods for the organic contaminants that are non-easily eliminated by biological
treatments. However, some flaws prevent their commercial applications such as the
high requirement of oxidant/catalyst dosage, high electrical power consumption, and
precise pH adjustment. Additionally, many previous studies also pointed out that
AOPs achieved a lower mineralization degree of the target compounds, and the
reaction products of the treatment process were higher toxic than the parent
compounds.

Thus, to overcome the above problems and to find efficient and economical
treatment, the combination of chemical and biological processes as a potential
alternative has been developed, in which the chemical treatment step has been applied
to improve the biodegradability of the wastewater by resulting in the intermediates,
which have been easily degraded in the subsequent biological treatment step. As
another kind of the autotrophic microorganism in the ecological system, microalgae
have been proved that have the capability to remove environmental contaminations
such as heavy metal, insecticides and other organic chemicals. There are also good
applications of the algae to treat antibiotics like tetracycline and norfloxacin. UV
irradiation has usually been combined with the biological methods to treat wastewater
Nevertheless, the potential of the algae combining UV irradiation to treat contaminants has been limited yet. Thus, the proposed system in the present study includes a UV irradiation step combined with an algal treatment step. The main objective of this study is to investigate the algal contribution in a combined UV-algae treatment to remove cefradine, a commonly and widely used antibiotic. The common freshwater alga species, *Chlorella pyrenoidosa*, was employed after the UV treatment. In several cases chemical oxidation increased the biodegradability of the target organic compounds accompanied by the toxicity increasing in the effluent while algae have proved to be a feasible approach to reduce the toxicity of organic compounds. Therefore, we evaluated and compared the removal capacity and effluent toxicity controlled to test our following hypothesis: (1) the performance of the individual algae treatment was inferior to the individual UV irradiation; (2) the algae treatment was necessary in a UV-algae combined treatment system to control the toxicity of the effluent after the UV treatment process. To better reveal the superiority of the algae, we also evaluate the algal contribution for the combined UV-activated sludge-algae treatment.

**Experimental set-up**

*Chemical and analytical method*

The antibiotic cefradine (>98% purity) used in the present work was purchased from Yabang investment holding group Co., Ltd. A high-performance liquid chromatograph (Beckman) equipped with an Inertsil ODS column (4.6 mm × 150 mm,
5 \mu m) was used to determine the concentration of the antibiotic. The mobile phase was as follows: water-methanol-3.86% sodium acetate-4% acetic acid (682:300:15:3). The flow rate was 1.0 mL min\(^{-1}\) under ambient temperature. The injection volume of the samples was 20 \mu L. All detections were performed by a UV detector at the wavelength of 254 nm. Quantization was performed using external standards and was based on peak areas. The limit of detection (LOD) and limit of quantification (LOQ) of the analytical methodology were determined based on 3.3 or 10 times the standard deviation of the background noise, respectively. The LOD and LOQ were 16.8 ng L\(^{-1}\) and 50.8 ng L\(^{-1}\), respectively. All removal rates in the following study were calculated using the transformed data from the HPLC signal. The total organic carbon content (TOC) of the samples was measured by TOC analyzer (Shimazdu TOC-L analyzer).

The green alga and the activated sludge culture

The common freshwater green alga \textit{C. pyrenoidosa} and the activated sludge were used in the biological treatment. The strain of the alga was obtained from the Institute of Hydrobiology of the Chinese Academy of Sciences. Cells were incubated in BG-11 medium and maintained at 25 ± 1 °C on a photoperiod 12h/12 h Light/Dark cycle under cool white fluorescent lights at an intensity of 40 \mu mol photons m\(^{-2}\) s\(^{-1}\). The activated sludge was collected from an industrial wastewater treatment plant (Nanjing, China) and cultivated for two weeks before the treatment at 25±1 °C in the dark.

Antibiotic Treatment process

In the present study, cefradine with an initial concentration of 150 mg L\(^{-1}\) was treated by three methods: the individual biological treatment (the algae treatment and
the activated sludge treatment), the individual chemical treatment (UV-irradiation) and the combined chemical-biological treatment (see Table 1). Schematic view of the technological process is presented in Fig. 1.

The individual algal treatment (Group 1) was performed in a 1 L Photo-reactors and conducted at 25±1 °C under a continuous illumination. After the pre-cultivation, the algae in exponentially growing phase were centrifuged and the algal pellets were re-suspended in a small volume of BG-11 medium and then diluted by the antibiotic solution. The initial algal density was about 10.00 × 10^6 cells mL\(^{-1}\). Samples were withdrawn from the reactor periodically to determine the algal density and the residual concentration of the target antibiotic at the given time during the treatment process. The individual activated sludge treatment (Group 2) was conducted in a 1 L cylindrical glass reactor by adding 450 mL of the antibiotic solution to 300 mL of the pre-cultured activated sludge, in which the actual sludge concentration was 2.20 ± 0.19 g L\(^{-1}\). The settling characteristics of the activated sludge in terms of sludge volume index (SVI) and mixed liquor suspended solid (MLSS) were detected before and after the treatment process. The experiments were conducted in the dark to prevent the possible photo-degradation of the target antibiotic.

The UV-irradiation treatment (Group 3) was carried out in a serpentiform quartz reactor. A high-pressure mercury lamp (500 W, E\(_{\text{max}}\)=365 NM, Xujiang Co., China) was placed in a water-jacked Pyrex tube that placed in the middle of the reactor. The time of UV-irradiation was controlled by a peristaltic pump installed at the outlet of the reactor which transfers liquids with alterable velocity. Samples were periodically
collected and filtered through 0.45 µm Millipore filters and then the concentration of the antibiotic was determined by HPLC. The combined chemical-biological treatment was divided into three groups (Group 4-6) that showed in Table 1. The solution with the initial cefradine concentration of 150 mg L\(^{-1}\) was treated by UV for 0.237 h to about 40 mg L\(^{-1}\). After filtered through 0.45 µm Millipore, the UV-treated solution was treated by the algae (Group 4) and the activated sludge (Group 5) for 24 h, respectively. In order to investigate the effect of initial antibiotic concentration, the cefradine at an initial concentration of 40 mg L\(^{-1}\) was also treated by the algae individually. In addition, the removal efficiency of the combined UV-activated sludge-algae treatment (Group 6) was also evaluated.

Toxicity assessment

The toxicity assessment of the treated effluents were performed following the guidance of ASTM, 2004 with some modifications. The test organism rotifer *Brachionus calyciflorus* was originated from a lake on the campus of China Pharmaceutical University (Nanjing, China). For the culture of the rotifer, the EPA medium contains 96 mg NaHCO\(_3\), 60 mg CaSO\(_4\)·2H\(_2\)O, 123 mg MgSO\(_4\)·7H\(_2\)O and 4 mg KCl in 1 L of deionized water with the pH adjusted to 7.5. The alga *C. pyrenoidosa* was offered as the diet. In the acute toxicity test, 10 juveniles (age of test animals < 24 h) were exposed to the test solutions in 24-well culture plates over a period of 24 h. The experiments were maintained at 25 ± 1 °C, with no food or light. The culture medium without any antibiotic was used as the control. At the end of the 24 h exposure, the number of dead rotifers was counted under the stereo microscope.
Rotifers were considered dead if no movement of the cilia and the mastax was observed over a period of 30 s. Each experiment had six replications per treatment.

Statistical analyses

All the data analyses were carried out with SPSS analytic package 21.0. Statistically significant differences among treatments were determined by analysis of variance (ANOVA). If the statistical test was significant at $p > 0.05$, a least significant difference (LSD) test was applied to find out where the difference occurred. All the figures were produced using Sigmaplot version 12.5.

Results and Discussion

To test whether the antibiotic could be treated by the individual biological treatment, the removal capacities of the individual algae and the activated sludge were compared (Fig.2). After a 24 h treatment of the activated sludge, 44.99% of cefradine were detected, which was lower than that treated by the algae (82.44% residue). In addition, even the initial concentration of the antibiotic was at 40.4 mg L$^{-1}$, high to 57.21% of cefradine was residual after the algae treatment. Our results indicated that the performance of the individual algae treatment was not effective to remove the antibiotic. Especially, the removal efficiency decreased significantly with the initial concentration of the target antibiotic increased. On the other hand, the activated sludge has been widely used in the wastewater treatments thanks to its high performance in dealing with large effluent flow rates and has been proved to be effective in dealing with some specific structured antibiotics. However, the removal
efficiency of the activated sludge is highly structure-dependent and can be variable between diverse microbial communities. Previous studies showed that $\beta$-lactams were more likely to be removed by the activated sludge treatment with an overall removal rate of cefoxitin (80%), cefotaxime (75%), and amoxicillin (81%), compared to the barely removal efficiency for ofloxacin (12%), respectively\textsuperscript{24}. While the high removal efficiency were not always occurred, especially for the polar and non-volatile antibiotics. Although there were several good applications to treat antibiotic. Our present results indicated that the activated sludge was not efficient in treating cefradine.

Though the individual biological treatment was inefficient in treating cefradine, we found that both of the algae and the activated sludge bacteria were tolerant to the impact of the target antibiotic. The algal population density increased during the treatment process (see Fig. 2.b), indicating that the green alga, \textit{C. pyrenoidosa} was tolerant to the antibiotic impact. In addition, the sludge concentration and the SVI changed from 2.19 to 2.21 g L\textsuperscript{-1} and 0.080 to 0.077 L g\textsuperscript{-1}, respectively (see Fig. 2d). The results suggested that the sludge had an excellent settling capacity and the sludge bulking did not occur. Considering the fact that the individual biological treatment could not remove cefradine effectively, additional treatment steps seem to be indispensable.

To better provide our hypothesis, the individual UV-treatment was also considered. The antibiotic cefradine at 150 mg L\textsuperscript{-1} was treated by the UV-irradiation at different velocities controlled by a peristaltic pump. After the UV-irradiation for 0.237
h, the antibiotic was degraded to 40.4 mg L$^{-1}$ rapidly. The first-order rate and the half-life of cefradine under the UV-irradiation was estimated to be 8.97 h$^{-1}$ ($r^2 = 0.98$) and 4.63 min, respectively, indicating that the antibiotic could be easily removed by the UV-irradiation. UV treatment is usual viewed to destroy the chemical bonds of a large variety of pollutants by direct photolysis in which the substance absorbs the photon directly and broke down into small molecule organic matters$^{25}$. Cefradine has a higher potential to undergo direct photolysis due to the light-sensitivity at 254 nm. Meanwhile, heavy metals iron, especially Fe$^{3+}$ containing in the alga culture and treatment medium may induce the generation of reactive oxygen species, thus resulting in indirect photolysis to the target antibiotic$^{26}$. Thus, the results provide our hypothesis 1 that the performance of the individual algae was inferior to the individual UV irradiation.

However, the change of the antibiotic concentration only indicated that the target antibiotic was non-detected, while the TOC decrease could give a measure of the mineralization degree of the target compound. Our results indicated directly that the removal rate of cefradine reached up to 73.07%, while only 5.39% of the TOC was removed (see Fig. 3a). High removal efficiency and low mineralization degree demonstrated that the target antibiotic was decomposed into small molecular weight organic matters rather than carbon dioxide, water or inorganic salts directly. The results were consistent with the previous studies$^{13}$. It's worth noting that many previous studies proved that the chemical treatment performed well in eliminating organic contaminant$^{12}$. However, the toxicity of the effluent remained or even
increased. Our study demonstrated that the effluent containing cefradine was sorely mineralized after the UV treatment. Thus, not only the removal efficiency was needed to evaluate, whether the UV-treatment increased the toxicity after the process should also be considered.

The rotifer acute toxicity assessment of the target antibiotic and its photo-degradation products was shown in Fig. 3b. Both of cefradine and its photo-degradation products showed a significant toxic effect on the rotifer \((p < 0.01)\). A high 24 h death rate \((93.33 \pm 8.16 \%)\) was calculated when the rotifer was exposed to the effluent after the UV treatment, which was 1.04 times than the parent compound \((90.00 \pm 8.94 \%)\). It indicated that the reaction products of the UV treatment process were higher toxic than the antibiotic itself. Comparing the corresponding change in the TOC, the increased toxicity of the antibiotic after the UV treatment might attribute to the generation of the by-products which were even less photo-sensitive than the parent compound. A similar result has been observed for oxytetracycline. The bacterial inhibition rate increased more than twofold after the UV-irradiation \((500 \text{ W}, \text{ E max}=365 \text{ nm})\) for 240 min. The UV photolysis system was applied to treat oxytetracycline, doxycycline and ciprofloxacin and the photoproducts still preserved the characteristic structure of the parent compounds, thus causing the toxicity increased. Thus, as for an appropriate treatment, not only the decay of the target compound should be evaluated, but also the toxicity of the treated water need to be considered.

Our above results indicated that although both of the algae and the activated
sludge bacteria survived under the impact of the antibiotic, the individual biological treatment was inefficient in the antibiotic removal process. On the contrary, the UV treatment reduced the antibiotic concentration efficiently, but increased the toxicity of the effluent. Considering the previous observation that the algae has the ability to reduce the toxicity of some organic contaminants, a UV-algae combined treatment was applied in the following step. In the combined treatment process, the UV-treatment step with the aim to improve the removal rate, while the algae treatment step with the aim to reduce the toxicity of the treated effluents. The total mass residual rate of the antibiotic which treated by the combined UV-algal treatment process was 22.01% (Fig. 4a), which was approximately a quarter of that treated by the individual algae treatment (82.44%).

In order to understand the detoxification ability of the algae, the acute toxicity of the effluents after 24 h algae treatment step in the present combined UV-algae treatment was detected. The death rate of the rotifers, which were exposed to the effluents after the combined UV-algae treatment was 55.00 ± 10.49% (Fig. 4b), significantly lower than that exposed to the effluents by the individual UV treatment (93.33±8.16%, see Fig.3b, \( p < 0.01 \)). It was obvious that when the algae treatment was used as the final step, the toxicity of the effluents could be decreased. Additionally, to better testify the algal contribution in detoxification, the toxicity control by the algae after the combined UV-activated sludge treatment was also evaluated. The removal efficiency of the combined UV-activated sludge treatment (17.81% residue) was better than that by the individual activated sludge treatment (see Fig.2), while
approaching the value occurred by the combined UV-activated sludge-algae treatment (16.01% residue). It indicated that the target antibiotic containing in the effluents after the combined UV-activated sludge process could barely be eliminated by the algae. In the toxicity assessment, about 40% of the tested rotifers died when they were exposed to the effluents by the UV-As treatment. However, a remarked toxicity control was obtained by the UV-As treatment which added an algal treatment step (23% of the tested rotifers died). Thus, the present results of the combined UV-activated sludge treatment and the combined UV-activated sludge-algal treatment could also confirm our hypothesis 2 that the role of the algae was to control the toxicity of the treated effluent.

The results described above emphasize that regarding treatment efficiency, UV is capable of destroying the antibiotic, but this is not necessarily accompanied by total mineralization or detoxification. The algae treatment contributed to the toxicity control in the effluent, while had a little fraction of the total removal efficiency. In the present combined treatment, the first stage was the degradation of the parent compound, causing the increasing of the toxicity of the by-products. The second stage was that these more toxic by-products were further converted into non-toxic byproducts. It implied that compared to the parent compound, the by-products might be utilized by the algae much easier. Thus, the decreased toxicity may be caused by the algal removal of the by-products. It has been observed that microalgae could grow heterotrophically under some specific conditions \(^ {27} \). When exposed to unfavorable living environment, such as nutrition deprivation and oxidative stress, the algae could
rapidly change in the metabolism pathway and intracellular components\textsuperscript{28}. For example, \textit{Chlorella sp.} IM-01 could efficiently uptake nitrogen and phosphorus from municipal wastewater as the nutrient source\textsuperscript{29}. Similarly, the presence of the microalgae \textit{Ulva lactuca} reduced the concentration of sulfathiazole efficiently by an efficient uptake capacity \textsuperscript{30}. \textit{Skeletonema costatum} was able to detoxify 2,4-dichlorophenol by conjugation to glutathione catalyzed by glutathione S-transferase \textsuperscript{31}. Up to now, most studies have comprehensively focused on discovering novel methods to remove antibiotic or analyzing the structure or the toxicity of the single degradation products. This study aimed to enhance the removal efficiency and the toxicity control of the effluent simultaneously. The combination of the UV and algae treatment has therefore been employed. In the process, the UV irradiation was effective in treating antibiotic, while the algae could control the toxicity of the effluents which became more toxic after the UV treatment. The green algae used in the present study is not the target organism of the antibiotic, which indicated that the alga also performed the population growth capacity under the impact of the antibiotic. After the destructive treatment by the UV irradiation, the small molecular organic compounds might serve as a carbon source and nitrogen source for the algae. Until now, information related to the metabolism of cephalosporin or the destructive compounds by microalgae has not been published. It is necessary to reveal the mechanism in further research, which might help us to better understand the algal contribution in the combined process. Further study will also be carried out to enhance the algal detoxification function by optimizing the algal culture
parameters, like the light source and the nutritional control.

**Conclusions**

In this study, we evaluated and compared the removal capacity of the individual algae treatment, the individual UV treatment and the combined UV-algae treatment to remove cefradine. The acute toxicity of the effluents after the above treatment processes on the rotifer was also assessed. To better reveal the detoxification function of the algae, we also compared with the corresponding properties of the combined UV-activated sludge-algae treatment and the combined UV-activated sludge treatment. The results showed that cefradine could be rapidly removed under the UV-irradiation (0.237 h -26.93% residue). Nevertheless, the toxicity of the effluents after the UV treatment higher than the parent compound. In the combined UV-algae treatment, the algal treatment step contributed to reduce the toxicity of the effluents from 93% to 55% (based on the death rate of rotifer). In the combined UV-activated sludge-algae treatment, the algal treatment step also conduced to further decrease the toxicity from 40% to 23% (based on the death rate of rotifer). Our present study indicated that the UV irradiation was responsible as a destructive treatment for the antibiotic and the algae treatment was necessary to control the toxicity of the effluent after the UV treatment process.

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References


13188-13196.


Table 1 Summary of the treatment process applied in the cefradine removal.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment process</th>
<th>Treatment Time (h)</th>
</tr>
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<tbody>
<tr>
<td></td>
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<td>UV</td>
</tr>
<tr>
<td>1</td>
<td>Algae</td>
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</tr>
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<td>2</td>
<td>Activated sludge</td>
<td>-</td>
</tr>
<tr>
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</tr>
<tr>
<td>6</td>
<td>UV-As-A</td>
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</tr>
</tbody>
</table>

Notes:

A: The algae treatment

As: The activated sludge treatment

UV: UV irradiation treatment

The initial concentration of cefradine was 150 mg L⁻¹
Fig. 1. Schematic view of the treatment process
Fig. 2. a: The total mass residual rate of cefradine at two concentrations (150 mg L\(^{-1}\) and 40.4 mg L\(^{-1}\)) during the individual algal treatment process; b: The algal density during the algal treatment process; c: The total mass residual rate of cefradine (150 mg L\(^{-1}\)) during the individual activated sludge treatment, d: The characteristics of the activated sludge before and after the treatment process.
Fig. 3. a: Cefradine removal and the TOC removal during the UV treatment; b: The death rate of the rotifer when exposed to the cefradine and its by-products after the UV treatment in 24 h.
Fig. 4. a: The total mass residual rate of cefradine during the combined UV-A treatment; b: The death rate of the rotifer when exposed to the effluents after the UV-A treatment.
Fig. 5. a: The total mass residual rate of cefradine during the combined UV-As treatment; b: The death rate of the rotifer when exposed to the effluents after the combined UV-As treatment; c: The total mass residual rate of cefradine during the combined UV-As-A treatment; d: The death rate of the rotifer when exposed to the effluents after the UV-As-A treatment.