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Short communication

Access the toxic effect of the antibiotic cefradine and its UV light degradation products on two freshwater algae

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ABSTRACT

Two common freshwater algae *Microcystis aeruginosa* and *Scenedesmus obliquus* were employed as test organism to evaluate the toxic effects of the widely used antibiotic, cefradine. In general, cefradine had significantly toxic effect on population growth and chlorophyll-a accumulation of two algae and the cyanophyceae was more sensitive than the chlorophyceae. In addition, cefradine UV light degraded products had adverse effect on *M. aeruginosa*'s growth and chlorophyll-a accumulation. In comparison, even if *S. obliquus* had growth ability when exposed to cefradine UV light-degradation products, the algal photosynthesis function was also disrupted.

1. Introduction

Chemicals released into the environment from human activities and agriculture are responsible for adverse ecological effects if the concentrations are higher than a threshold of environmental self-purification and organism tolerance [1]. Antibiotics are defined as chemical compounds that inhibit the growth of other microorganisms [2]. As several hundred different antibiotic substances are used in human and veterinary medicine in the last years, the cases of surface water contamination by antibiotics have been reported since 1982 [3–6]. Antibiotics have been considered emerging pollutants due to their continuous input and persistence in the aquatic ecosystem. The substances are only partially eliminated in sewage treatment plants. If they are not eliminated during the purification process, they pass through the sewage system and may mainly end up in the water compartment. Those used in aquaculture directly will also reach surface water where they may affect the aquatic organisms [7]. Alga is the important component of the primary production and detrimental effects in these organisms may affect the entire food chain [8]. A very low concentration of pollutants in the water could exert detrimental influences on algae [1]. Study on the algal toxicity of antibiotic should help us to better understand how the substances affect non-target organisms, disrupt the food chain, modify the food web and change the interspecific interactions between algae, which cause an imbalance in the entire ecosystem.

However, the reported ecotoxicological researches of the antibiotics on algae were mainly focused on β-lactam class, such as amoxicillin, mecillinam and benzyl penicillin, macrolides class, spiramycin, quinolone class, such as fluoroquinolone, sarafloxacin and ciprofloxacin and nitroimidazole class, metronidazole [9–13]. Alga toxicity tests of the widely used antibiotic, cefradine were limited. Therefore, the aim of this research is to assess the toxic effect of cefradine on freshwater algae *Microcystis aeruginosa* and *Scenedesmus obliquus*, not only on population growth, but also on algal physiological function. In addition, because the substance could be light sensitive, the toxic effect of cefradine and its UV light degradation was compared in our study, which were needful but neglected in the past.

2. Experimental

2.1. Test organisms and culture condition

Strains of the freshwater cyanophyceae *M. aeruginosa* (FACHB-1005) and the chlorophyceae *S. obliquus* (FACHB-416) were obtained from the Institute of Hydrobiology of the Chinese Academy of Sciences. *M. aeruginosa* was cultured in BG-11 medium, while *S. obliquus* was cultured in SE medium. The two algae were incubated and maintained at 26 ± 1 °C under an illumination intensity of 2000 lux, with a 12 h/12 h light/dark interval.

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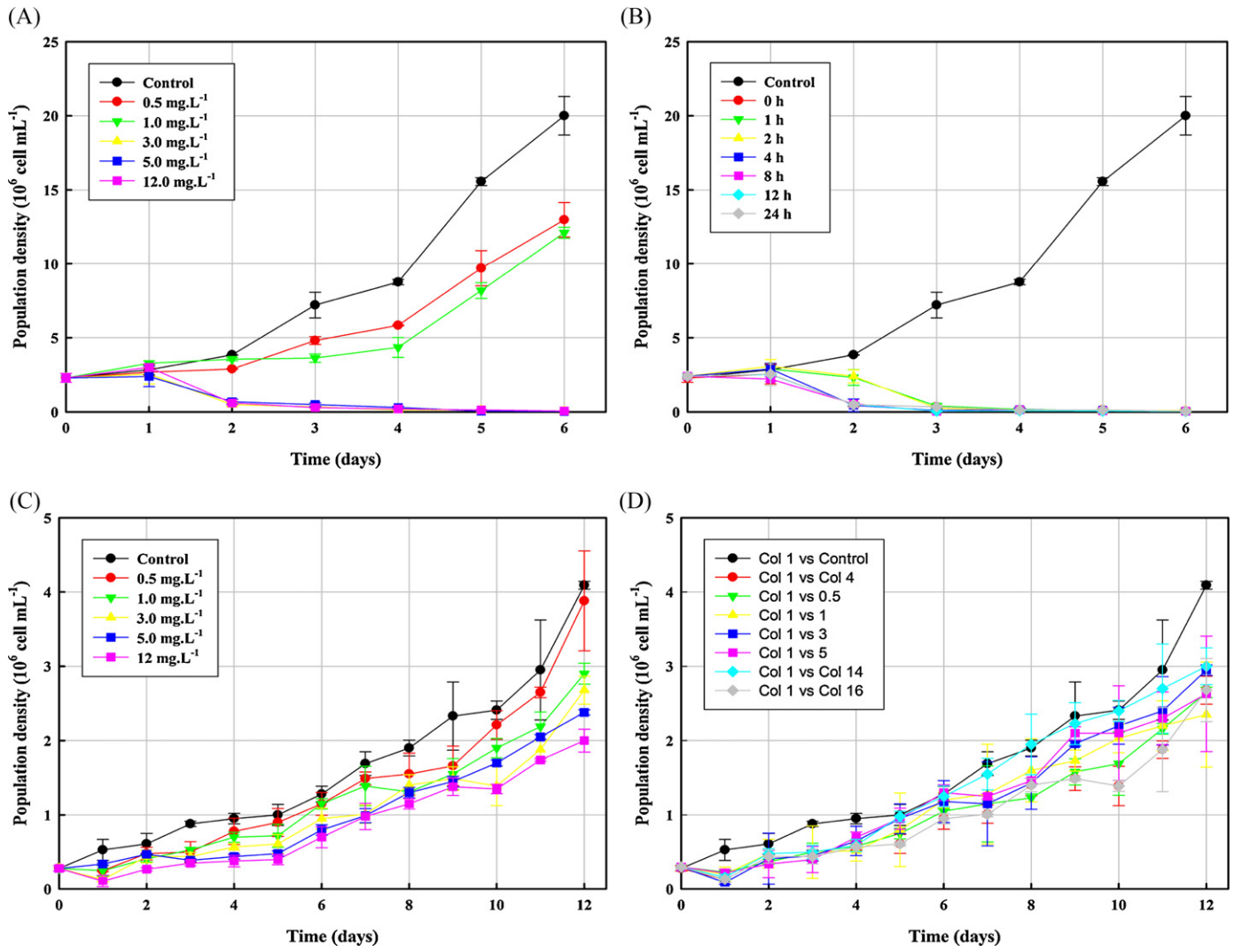

Fig. 1. Population growth curves of two freshwater algae *Microcystis aeruginosa* and *Scenedesmus obliquus* cultured under cefradine. A: *M. aeruginosa* under five concentrations of cefradine, B: *M. aeruginosa* under cefradine with different time of UV light degradation treatment, C: *S. obliquus* under five concentrations of cefradine, D: *S. obliquus* under cefradine with different time of UV light degradation treatment.

2.2. Toxicity assays

The algal inoculum was prepared for each experiment from fresh culture stocks sampled during the exponential growth phase. Toxicity assays were performed into two parts: antibiotic toxicity test (Experiment *I*) and antibiotic UV light degraded products toxicity test (Experiment *II*). Experiments were continued 6 and 12 d respectively (fixed by preliminary tests in order to research the maximum population increase rate of two algae respectively). In Experiment *I*, an equal volume of fresh culture medium of 100 mL in the presence of the antibiotic at various concentrations was added to the algal pellets in 250 mL of previously sterilized conical flask. The corresponding cefradine concentrations were 0.5, 1, 3, 5 and 10 mg L^{-1}. In Experiment *II*, the cefradine in median concentration (3 mg L^{-1}) was degraded under UV light in 0, 1, 2, 4, 8, 12 and 24 h. The algae cultured without any pollutant was used as control. All cultures were cultivated followed the culture condition described above. Samples were removed from the culture vessels at predetermined times every day. The cells were observed microscopically using hemacytometer. The population growth rate (r) was calculated from the formula: $r = 1/t(\ln N_t - \ln N_0)$; where N_t and N_0 are population sizes at day 0 and day t, and t is time in days when the population size is maximum [14]. Chlorophyll-a content was analysed using standard method [15]. Each experiment had three replications per treatment.

2.3. Statistical analyses

All the data analyses were carried out with the SPSS analytic package 16.0. Data were first tested for homogemeity (Levene's test). Variables from the results in experiment were examined by one-way analysis of variance (ANOVA) to identify significant differences. All the figures were produced using Sigmaplot Version 11.0.

3. Results and discussion

3.1. Cefradine toxicity test

For *M. aeruginosa*, when the cefradine concentrations were lower than 3.0 mg L^{-1}, the population increased until the end of the experiment (Fig. 1A). The maximum population sizes were 12.98 and 12.11 × 10^6 cell mL^{-1} in average at 0.5 and 1.0 mg L^{-1}, respectively, which were 64.90% and 60.55% of that in control (20.00 × 10^6 cell mL^{-1} in average). However, the population dynamics at higher concentrations (≥3.0 mg L^{-1}) indicated that

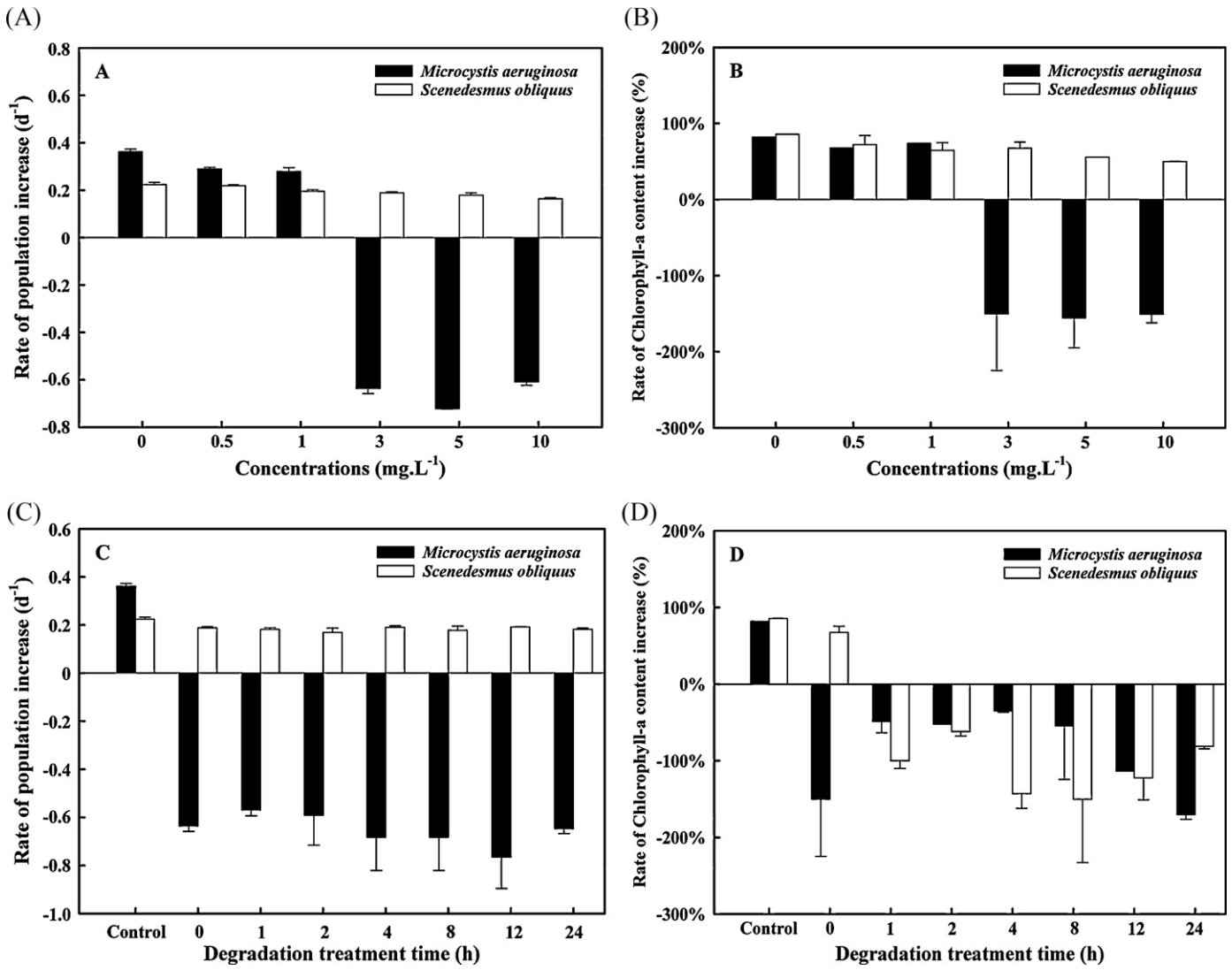

Fig. 2. The rate of population increase (d^{-1}) and the rate of chlorophyll-a content increase (%) of the two freshwater algae *M. aeruginosa* and *S. obliquus* cultured under cefradine. A: the rate of population increase of two algae under five concentrations of cefradine, B: the rate of chlorophyll-a content increase of two algae under five concentrations of cefradine, C: the rates of population increase of two algae under cefradine with different time of UV light degradation treatment, D: the rate of chlorophyll-a content increase of two algae under cefradine with different time of UV light degradation treatment.

cefradine produced a negative influence on the growth of *M. aeruginosa*. In general, the cyanophyceae population declined since the second day and a large number of dead algal cells were observed. The rates of algal population increase were all lower than 0 (negative value). The rate of chlorophyll-a content increase (%) under given concentrations are presented in Fig. 2B. Similar trends about the influence of cefradine on algal physiological function were observed clearly. The chlorophyll-a was accumulated during 6 d (positive increase rate), even though the contents increase rates were less than that in control when concentrations at 0.5 and 1.0 mg L^{-1}, while it was inhibited powerfully when concentrations were higher than 3.0 mg L^{-1}. In comparison, for *S. obliquus* cultured exposed to cefradine in five concentrations, although the population increased during the whole experiment time under any given cefradine concentration, the maximum population sizes were 3.88, 2.90, 2.68, 2.38 and 2.00×10^6 cell mL^{-1} in average, respectively, which were 94.87%, 70.90%, 65.53%, 58.19% and 48.90% of that in control (Fig. 1C). The trends about the influence of cefradine were observed clearly. The rate of population increase and chlorophyll-a content declined with an increase in concentrations (Fig. 2A and B).

The population growth curves of the two freshwater algae cultured under cefradine with different time of UV light degradation treatment are presented in Fig. 1B and D. Compared to that in control, it was indicated that the UV-degraded cefradine produced a significantly negative influence on the growth of *M. aeruginosa* ($F_{7,23} = 44.42$, $p < 0.01$, *F*-test). The cyanophyceae population declined since the second day and a large number of deal algal cells were observed after then. The rates of algal population increase and chlorophyll-a content increase were all negative under any given concentration (Fig. 2C and D). In comparison, the population of *S. obliquus* increased during 12 d, regardless of the degradation time, even if the population densities were lower than that in control (Fig. 2C). In addition, the differences among the population increase rate in given concentration were significantly lower than that in control ($F_{5,16} = 27.96$, $p < 0.01$, *F*-test). However, the rates of chlorophyll-a content increase were all negative under any given degradation time (Fig. 2D).

The sensitivity of algae towards antibiotics varies widely. For example, *Selenastrum capricornutum* was found to be two to three orders of magnitude less sensitive to most antibiotics than *M. aeruginosa* in toxicity test. The growth of *M. aeruginosa* was

inhibited by several antibiotics such as benzylpenicillin, chlortetracycline, spiramycin, streptomycin, tetracycline, tiamulin and tylosin at concentrations of less than 0.1 mg L^{-1} [11]. Boxall [9] pointed that blue-green algae (cyanobacteria) seem to be sensitive to many antibiotics. Similar trends were observed in our study. 72h-EC_{50} values of two algae were 1.38 (*M. aeruginosa*) and 1.77 (*S. obliquus*) mg L^{-1}, which showed that *M. aeruginosa* was more sensitive than *S. obliquus.* In addition, the cyanophyceae population declined since the second day and a large number of deal algal cells were observed when the cefradine concentrations were higher than 3.0 mg L^{-1}, while *S. obliquus* also had weak growth ability under any given concentration. Although the growth inhibition curves of *Chlorella vulgaris* under surfactants in 12 days was presented recently [16], similar result about antibiotics was not reported. In our study, it is indicated that cefradine had stronger adverse effect on *M. aeruginosa* than on *S. obliquus* with the concentrations increased. Different antibiotics have different chemical structures and therefore have varied mechanism of action. It is possible that some antibiotics are highly toxic for the alga, while are not likely to affect other alga adversely. For this reason, it is hard and not necessary to compare the toxicity test results in different research. But all the results indicated that potential adverse effects of antibiotics on algae could not be excluded.

3.2. UV-degraded cefradine toxicity test

Light-decomposition may be of major significance in the elimination process if a substance is light sensitive [7]. It should be noted that incomplete photo-degradation could lead to more or less stable or more or less toxic products [17]. Unfortunately, few studies focused on the toxicity of the light-degradation products from antibiotic. Consequently, the algal toxicity of cefradine UV light-degradation products were tested in our study for a better understanding of the behavior of antibiotic in the environment, especially the risks associated with their occurrence. For *M. aeruginosa*, regardless of the UV light degradation time, the treated cefradine also had adverse effect on algal growth and chlorophyll-a accumulation (Fig. 2C and D, black bars). However, the linear-correlation between the toxicity and degradation time was not clear (R^2 = 0.49 and 0.01, respectively for algal growth and chlorophyll-a accumulation) and toxicity enhanced in fact had not followed the increase or decrease in degradation time. In comparison, similar trends of *S. obliquus* about nonlinear-correlation between the toxicity and degradation time has been presented in Fig. 2D (white bars). In general, the UV-degraded cefradine under any treatment time was more toxic for the algal chlorophyll-a accumulation than cefradine itself excluding under 24 h. Nonetheless, response of chlorophyll-a accumulation under cefradine UV light degradation products should be payed more attention. Fig. 2C and D showed that although *S. obliquus* grew better than *M. aeruginosa* when exposed to cefradine under any degradation treatment time, the algal chlorophyll-a accumulation were also inhibited. Our result suggested that even if *S. obliquus* had growth ability when exposed to cefradine UV light degradation products (Fig. 2C, white bars), the algal photosynthesis function was also disrupted. The cefradine UV degraded segments could cause different degree of toxicity on algae in mixtures, due to their potential combined, synergistic or antergic action. It might be explained the results in our study that toxicity enhanced on two algae did not follow the increase or decrease in degradation time. It is therefore necessary to identify, isolate and characterize the segments and assess their toxic effects in future.

4. Conclusions

The antibiotic cefradine had significantly toxic effect on *M. aeruginosa* and *S. obliquus*. Compared with the data in two algae, the cyanophyceae was more sensitive than the chlorophyceae. In addition, cefradine UV light degraded products had adverse effect on *M. aeruginosa*'s growth and chlorophyll-a accumulation. Although *S. obliquus* had growth ability when exposed to cefradine light-degradation products, the algal photosynthesis function was also disrupted.

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