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IJESP International Journal of Environmental Sustainability and Protection

ISSN 2737-5447
Volume 2, Issue 3
https://www.iikii.com.sg/journal/IJESP
International Journal of Environmental Sustainability and Protection

Article

Effects of Two Quinolone Antibiotics on Growth of Four Species of Planktonic Algae

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Received: Aug 8, 2022; **Accepted:** Sep 8, 2022; **Published:** Sep 30, 2022

Abstract: Quinolone antibiotics, especially enrofloxacin and ciprofloxacin, are currently found to be at high levels commonly in natural waters. Studying the effects of these two quinolones on the growth of common microalgae in water is valuable for understanding the ecological effects of quinolones in the aquatic environment. Therefore, the effects of different concentrations of enrofloxacin and ciprofloxacin hydrochloride on the population growth of four species of planktonic microalgae (*Chlorella vulgaris*, *Tetradesmus obliquus*, *Scenedesmus quadricauda*, and *Microcystis aeruginosa*) were studied. The results showed that there was a significant effect-dose regression relationship between the concentration of nofantibiotic and the growth inhibitory rate of the algae population. The 96h half maximal inhibitory concentration (96h-IC50) of enrofloxacin on *T. obliquus*, *S. quadricauda*, *M.aeruginosaI* and *C. vulgaris* were 195.6, 88.8, 56.1, and 22.6 mg/L respectively, and the order of sensitivity of the four microalgae to enrofloxacin was *T. obliquus* < *S. quadricauda* < *M. aeruginosa* < *C. vulgaris*. Another 96h-IC50 of ciprofloxacin hydrochloride to *S. quadricauda*, *T. obliquus*, *M. aeruginosa*, and *C. vulgaris* were 588.6, 546.3, 49.8, and 44.7 mg/L respectively. The sensitivity of the four microalgae to ciprofloxacin hydrochloride was ranked as follows: *S. quadricauda* < *T. obliquus* < *M. aeruginosa* < *C. vulgaris*. Among the four species of planktonic microalgae, two species of *Scenedesmus* were less sensitive to the quinolone antibiotics. Therefore, these two species of *Scenedesmus* were more resistant to the two quinolone antibiotics, while *C. vulgaris* was the most sensitive to them. According to the data of IC50, the toxicity of enrofloxacin to three green microalgae was higher than that of ciprofloxacin, while the toxicity of enrofloxacin to *M. aeruginosa* was lower than that of ciprofloxacin. The population growth of the four planktonic microalgae was inhibited in the first 4 days after being treated by these two quinolone antibiotics, but the microalgae could gradually detoxify and restore their population growth after 96 h. Moreover, the toxicity of enrofloxacin to the four algae increased with time. The toxicity of ciprofloxacin hydrochloride to *T. obliquus* and *S. quadricauda* increased, and the toxicity to *M. aeruginosa* and *C. vulgaris* decreased. The recovery degree of ciprofloxacin hydrochloride was at least 39% higher than that of enrofloxacin. Low concentrations of enrofloxacin (<36 mg/L) provoked a higher density of *M. aeruginosa* than that of the control group after 10 days of exposure. In this case, the algal density of *M. aeruginosa* could be more than 10^7 cells/L when the concentration of enrofloxacin was below 36 mg/L.

Keywords: Enrofloxacin, Ciprofloxacin, *Chlorella vulgaris*, *Tetradesmus obliquus*, *Scenedesmus quadricauda*, *Microcystis aeruginosa*, toxicity, Half maximal inhibitory concentration

1. Introduction

Quinolone antibiotics had been widely used in disease prevention of humans, livestock, and aquaculture animals due to their broad antibacterial spectrum, strong antibacterial activity, no cross-resistance with other antimicrobials, and low side effects [1]. According to statistics, the annual output of quinolones in China is about 700 tons, of which more than half are used in aquaculture [2]. These antibiotics widely used in livestock breeding and aquaculture can accumulate in animals such as livestock, fish, and shrimps and may lead to drug resistance of bacteria. Once residual antibiotics and drug-resistant bacteria enter the human body, it may affect human health [3]. At present, more than 50 kinds of antibiotics have been detected in water bodies[4], while enrofloxacin and ciprofloxacin hydrochloride have a high detection rate and are the most common antibiotics detected in aquatic products [5]. Moreover, the residue of enrofloxacin in aquatic products has exceeded 50 μg/kg (the Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO)) with a detection rate of 46.9% and the maximum residue of 67 μg/kg [6–

9]. These two antibiotics had been widely used in aquaculture for a long time due to a lack of necessary drug management and scientific guidance. Thus, the phenomenon of antibiotic abuse is widespread, and the problems of aquatic product quality and ecological safety by such antibiotics have attracted more attention, restricting the healthy and sustainable development of aquaculture.

At present, due to the lack of systematic and in-depth studies on the pharmacology and ecotoxicology of commonly used fishery drugs, the evaluation of the ecological effects on aquatic organisms are not clear enough [10]. The research on quinolone antibiotics in aquaculture mainly focuses on their pharmacodynamics and pharmacokinetics on pathogenic bacteria [11–13]. Nowadays, scholars pay more attention to the effects of various drugs on non-target organisms in the ecosystem [14]. Freshwater planktonic microalgae are the main primary producers in the aquatic food chain [15] and also are important indicator organisms for monitoring and evaluating water environment quality and pollutant ecotoxicity [16]. Freshwater planktonic microalgae are high-quality live prey food for economic aquaculture animals and play an important role in maintaining the balance and stability of the water ecosystem [17]. It has been reported that antibiotics can affect the metabolic activities of freshwater planktonic microalgae and the growth and physiological activity of freshwater planktonic microalgae [18–20]. The effects of exogenous pollutants on algal growth can be useful for evaluating aquatic ecosystem [21], Therefore, freshwater planktonic microalgae are ideal organisms to detect the toxicity of water pollutants such as antibiotics, so it is of great theoretical and practical significance to study the effects of quinolone antibiotics on the population growth of freshwater planktonic microalgae. Accordingly, this study is carried out to investigate the effects of two quinolone antibiotics (enrofloxacin and ciprofloxacin) on the growth of four common freshwater algae (*C. vulgaris*, *T. obliquus*, *S. quadricauda*, and *M. aeruginosa*) to provide a reference for ecological risk assessment in water.

2. Materials and Methods

2.1. Test Material

- Algal species

C.vulgaris (FACHB-2338), *T. obliquus* (FACHB-12), *S. quadricauda* (FACHB-44), and *M. aeruginosa* (FACHB-930) were purchased from the Freshwater Species Bank of the Institute of Hydrobiology, Chinese Academy of Sciences, and cultured for several generations in our laboratory.

- Test drugs

Enrofloxacin injection, with a purity of 5%, was produced by Shanghai Tongren Pharmaceutical Co., Ltd. Shanghai Veterinary Drug Factory and ciprofloxacin hydrochloride injection, purity 5%, produced by Jiangxi Heguang Pharmaceutical Co., Ltd were used in the experiment. Algal culture liquid was BG11 liquid medium [22].

2.2. Test Method

- Algae culture

Before culturing algae, the culture medium and containers were autoclaved at 121°C for 30 min, and then the relevant experimental supplies were transferred to the sterile operation table to wait for the medium to cool and UV-sterilize for 30 min before the subsequent operation. Under aseptic conditions, the four microalgae were cultured in BG11 medium until the logarithmic growth stage which further expanded. The culture condition was a diurnal static culture under a natural light-dark ratio for 12 h and a temperature of 25–34 °C. The cells were manually shaken three times a day at regular intervals to reduce algal cell apposition. After the microscopic examination of the cells was normal, the algae at the time of entering the logarithmic growth phase were taken for testing [23].

- Standard curve

Based on the conditions and accuracy of the test, the standard curve for the absorbance value at 680 nm wavelength versus algal cell density was used to calculate the algal cell density [24]. The specific method is as follows.

(1) 1, 3, 5, 10, 15, and 20 mL of algal solution were put in 6 beakers with a measuring cylinder, and deionized water was added to fix the volume to 25 mL to obtain different densities of algal solution. A small amount of algal solution was aspirated, and the algal cells in the samples were counted accurately under a microscope using a 0.1mL phytoplankton counting frame. Each group of samples was counted three times and the average value was taken.

(2) The absorbance values of different densities of algal solutions were measured at 680 nm with a UV spectrophotometer with deionized water as the reference, and each sample was measured three times and the average value was taken.
(3) The measured data were used to establish the standard curve between absorbance values and algal cell density in Excel.

- Toxicity test

Before the formal test, a preliminary experiment is performed to determine the mass concentration range required for the formal test. A 20 mL test tube was taken and added with a certain amount of ciprofloxacin hydrochloride, enrofloxacin injections, and algae solution so that the IC50 value at 96 h was included [25]. In the concentration range determined by the pre-experiment, 4 concentration values were selected as the exposure dose of the formal experiment, and a blank control group was set at the same time. The concentration gradients of the two antibiotics to the four algae were set as follows (Table 1).

Table 1. Antibiotic concentration gradient.

Algae Species / Antibiotic Concentration (mg/L)	*Chlorella vulgaris*	*Tetradesmus obliquus*	*Scenedesmus quadricauda*	*Microcystis aeruginosa*
enrofloxacin	0	0	0	0
	24	60	60	12
	48	120	90	36
	96	240	120	72
	120	480	240	120
ciprofloxacin hydrochloride	0	0	0	0
	15	150	600	30
	30	300	900	45
	45	450	1200	60
	60	600	1500	150

When 75 mL of algal liquid was in the logarithmic growth phase, the two antibiotics were added according to the concentrations in Table 1 to set up three parallel groups for each concentration and measure the initial absorbance. The microalgae were routinely cultured, and the absorbance of microalgae concentration was measured at 24, 48, 72, 96, 168, 240, and 336h. The growth inhibition rate was then calculated.

2.3. Data Processing

The reduction in the number of cells in the treated group compared to the untreated control group is expressed as cell growth inhibition, while drug sensitivity is usually expressed as the drug concentration at which cell growth inhibition reaches 50%, i.e. IC50 (50% inhibitory concentration).

- Growth inhibitory rate

The calculation formula of the growth inhibitory rate is

$$\text{growth inhibition rate} = (1 - N/N_0) \times 100\% \quad (1)$$

where N is the concentration of algae in the experimental group, 10^5 /mL, and N_0 is the concentration of algae in the control group, 10^5 /mL.

- Half inhibitory concentration (IC50)

The data were statistically analyzed by Boltzmann constant fitting in Origin 2021 software to analyze the sensitivity of green algae at different concentrations of antibiotics. At the same time, a nonlinear function model was used to perform nonlinear fitting on the 96h growth inhibition data of algal cells, and then the relevant parameters of each fitting model were obtained. The effect value of a single antibiotic on the concentration gradient is obtained by fitting the nonlinear function of Boltzmann [26]. The function formula is as follows.

$$Y = A_2 + (A_1 - A_2) / (1 + e^{(X - X0)/dx}) \quad (2)$$

where X represents the antibiotic concentration, Y represents the inhibitory rate of microalgae production, dx is the slope parameter, X_0 is the center point of the curve, and A_1 and A_2 are the upper and lower asymptotes. With Eq. (2), the IC_X of each antibiotic on the algae [27] was obtained to quantitatively analyze the effect of antibiotics on algae growth.

3. Results

3.1. Linear Relationship between Algal Cell Density and Absorbance Value

The density of algal cells was calculated after measuring the absorbance value according to the regression equation. The regression curve of the four algal cell densities and the absorbance value at 680 nm was shown in Fig. 1. R^2 of them were all greater than 0.99, indicating a good linear relationship between them.

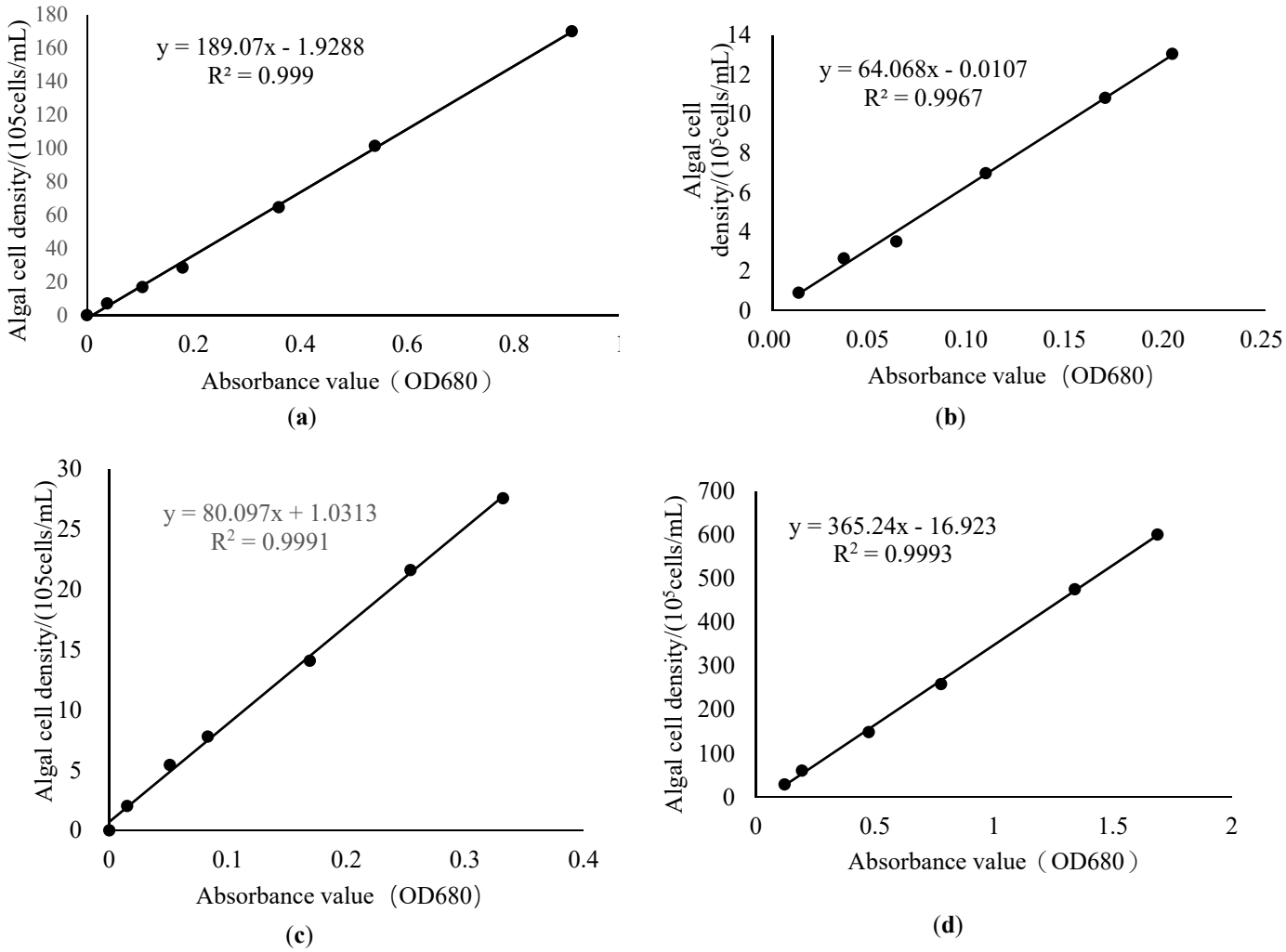


Fig. 1. (**a**) Linear relationship between cell density and absorbance values of *C. vulgaris*; (**b**) Linear relationship between cell density and absorbance values of *T. obliquus*; (**c**) Linear relationship between cell density and absorbance values of *S. quadricauda*; (**d**) Linear relationship between cell density and absorbance values of *M. aeruginosa*.

3.2 Effects of Two Antibiotics on Growth of Algal Cells

3.2.1. Effects of Enrofloxacin on Growth of Planktonic Microalgae

The growth effect kinetic curve of the four microalgae cells treated with enrofloxacin at different concentrations is shown in Fig. 2. Compared with the blank group, different concentrations of enrofloxacin had different inhibitory effects on algal cells. The inhibitory effect on algal density increased with the increase of enrofloxacin concentration. Within the first 96h, the density of algal cells in each experimental group increased limitedly, but except for *M. aeruginosa*, the densities of the algae were lower than the

blank control group. With the extension of time, the growth rate did not increase significantly, and all showed negative growth at 96h. In contrast, although the experimental groups with the four concentrations were completely inhibited in the first 96 h and the growth rate was close to 0, the inhibition did not increase significantly with the increase of the concentration. In the process of pre-experiment, it was found that a low concentration of enrofloxacin promoted the growth of four algae, and *M. aeruginosa* showed promoted growth and then inhibited growth under 12 mg/L enrofloxacin, which shows that enrofloxacin needed to accumulate to a certain amount in algal cells to cause damage to the cells.

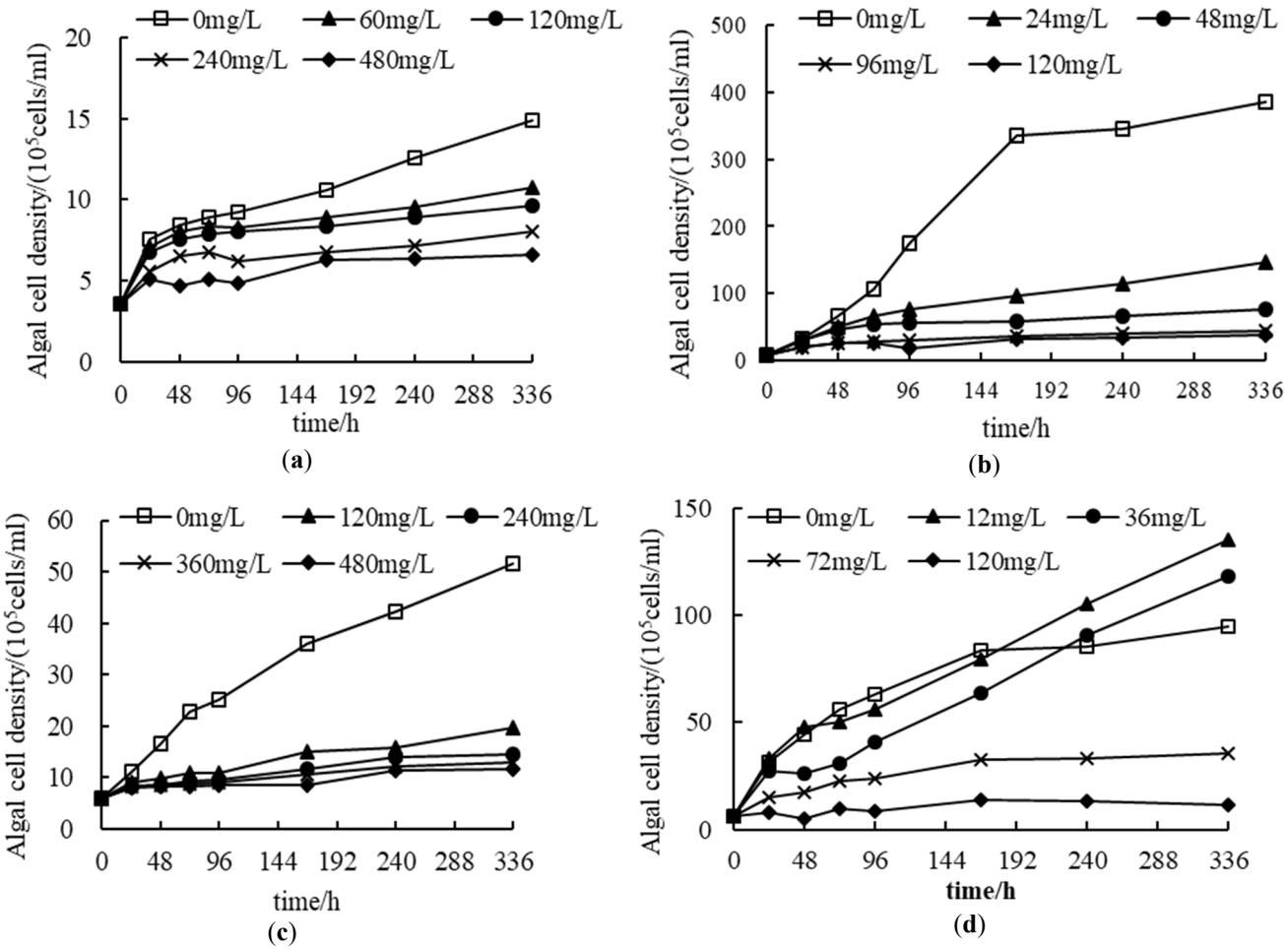


Fig. 2. (**a**) Kinetic curves of the effect of enrofloxacin on the growth of *C. vulgaris*; (**b**) Kinetic curves of the effect of enrofloxacin on the growth of *T. obliquus*; (**c**) Kinetic curves of the effect of enrofloxacin on the growth of *S. quadricauda*; (**d**) Kinetic curves of the effect of enrofloxacin on the growth of *M. aeruginosa*.

At 96 and 336 h, the percentage of the algal density of *T. obliquus* in the lowest concentration group (24 mg/L) and the control group (CG) was 89 and 72%, respectively. The highest concentration group (120 mg/L) and CG had 52 and 44%, respectively. The population growth of *T. obliquus* was inhibited continuously at a whole duration of 336h when it was treated with enrofloxacin. The percentages of the algal density of *S. quadricauda* in the lowest concentration group (120 mg/L) and CG were 44 and 38%, respectively. The highest concentration group (480mg/L) and CG were 32 and 22%, respectively (Table 3). The population growth of *S. quadricauda* was also inhibited at a whole duration of 336 h when it was treated with enrofloxacin**.**

Table 4 shows that at 96 h and 336 h, the percentage of the algae density of M. *aeruginosa* in the lowest concentration (12mg/L) group and CG was 89 and 143%. M. *aeruginosa* in the group with 12 and 36mg/L at 336 h showed that the algal density was higher than in the blank group. The result implies that M. *aeruginosa* could detoxify below the concentration of 36 mg/L of enrofloxacin at the duration of 336h. The percentage of algae density in the highest concentration (120 mg/L) group and CG was only 14 and 12%, showing that M. *aeruginosa* cannot detoxify above a concentration of 72 mg/L of enrofloxacin at the duration of 336h.

C. vulgaris at 96 and 336h had the percentage of algae density in the lowest concentration (60 mg/L) and CG as 44 and 38%. The percentage of the algae density in the highest concentration (480 mg/L) and CG was 11 and 10%. The population growth of *C. vulgaris* was inhibited continuesly at a whole duration of 336h when it was treated with enrofloxacin.

Table 2. Cell density of *T. obliquus* after treatment with different concentrations of enrofloxacin.

Concentration(mg/L)	96 h	336 h	96 h	336 h
	Alage cell density(10^5 cells/mL)		Percentage of the CG	
Control group(CG)	9.247	14.853	-	-
24	8.318	10.746	89%	72%
48	8.081	9.651	87%	65%
96	6.249	8.068	67%	54%
120	4.871	6.588	52%	44%

Table 3. Cell density of *S. quadricauda* after treatment with different concentrations of enrofloxacin.

Concentration(mg/L)	96 h	336 h	96 h	336 h
	Alage cell density (10^5 cells/mL)		Percentage of the CG	
Control group(CG)	25.261	51.693	-	-
120	11.027	19.710	44%	38%
240	9.522	14.608	40%	28%
360	9.081	12.862	36%	25%
480	8.464	11.564	32%	22%

Table 4. Cell density of *M. aeruginosa* after treatment with different concentrations of enrofloxacin.

Concentration(mg/L)	96 h	336 h	96 h	336 h
	Alage cell density (10^5 cells/mL)		Percentage of the CG	
Control group(CG)	63.247	94.658	-	-
12	56.198	135.309	89%	143%
36	41.077	118.143	65%	125%
72	23.911	35.306	38%	37%
120	8.717	11.310	14%	12%

Table 5. Cell density of *C. vulgaris* after treatment with different concentrations of enrofloxacin.

Concentration(mg/L)	96 h	336h	96 h	336 h
	Alage cell density (10^5 cells/mL)		Percentage of the CG	
Control group(CG)	174.275	385.364	-	-
60	76.294	146.169	44%	38%
120	56.866	77.094	33%	20%
240	29.464	43.508	17%	11%
480	18.751	38.560	11%	10%

3.2.2. Effects of Ciprofloxacin Hydrochloride on Growth of Planktonic Microalgae

The kinetic curves of the growth effect of the algal cells treated with different concentrations of ciprofloxacin hydrochloride are shown in Fig. 3. With enrofloxacin, there was a significant dose-response relationship between ciprofloxacin hydrochloride concentration and the rate of algal cell growth inhibition. Compared with the blank group, different concentrations of ciprofloxacin hydrochloride had different inhibitory effects on algal cells, and the inhibitory effect on algal density increased with the increase of ciprofloxacin hydrochloride concentration.

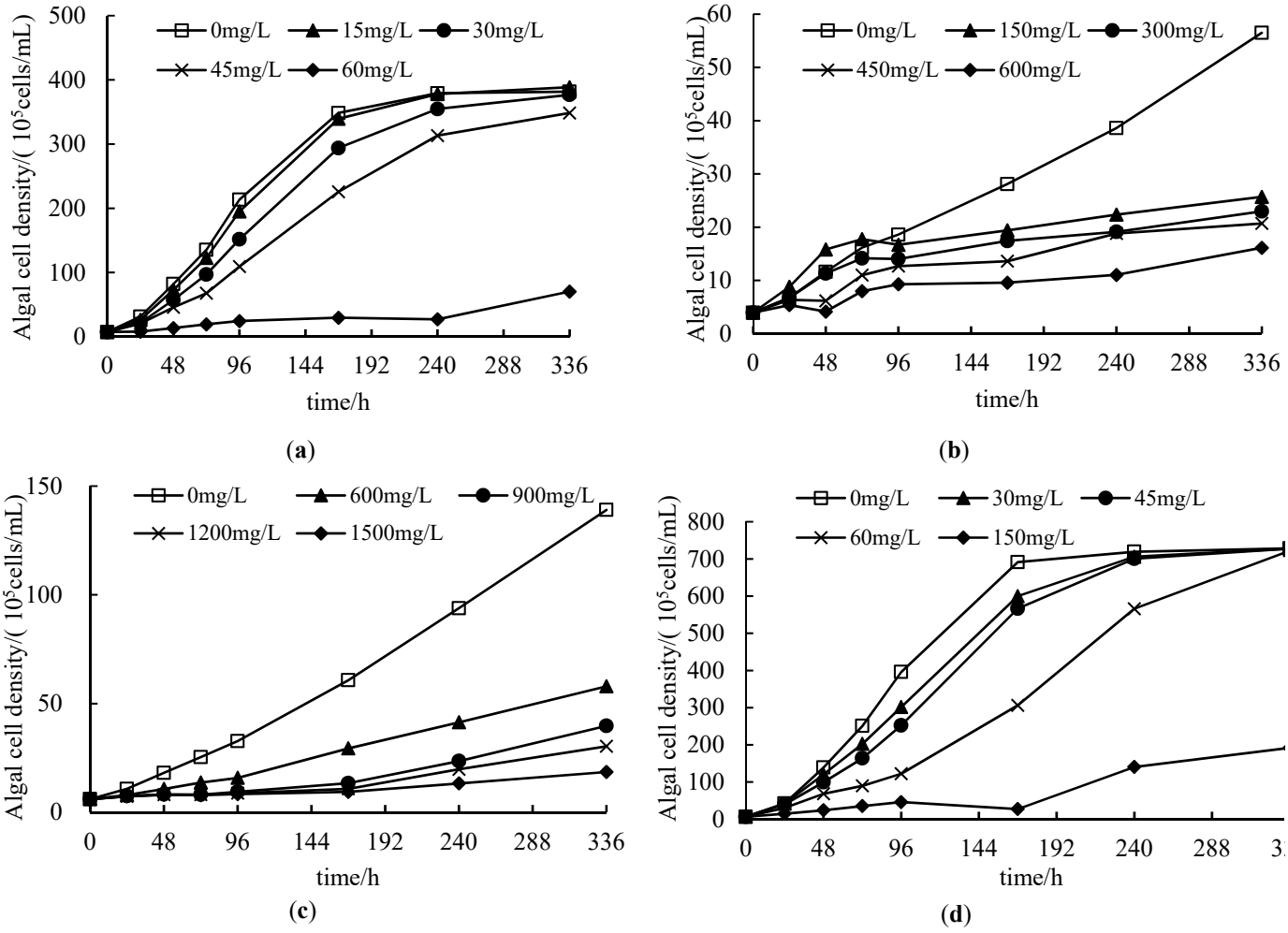


Fig. 3. (**a**) Kinetic curves of the effect of ciprofloxacin hydrochloride on the growth of *C. vulgaris*; (b) Kinetic curves of the effect of ciprofloxacin hydrochloride on the growth of *T. obliquus*; (**c**) Kinetic curves of the effect of ciprofloxacin hydrochloride on the growth of *S. quadricauda*; (**d**) Kinetic curves of the effect of ciprofloxacin hydrochloride on the growth of *M. aeruginosa*.

Within the first 96 h at the set concentration, the high-concentration group was almost completely inhibited except for *T. obliquus*. The growth of the cells in the low-concentration group increased but was lower than that of the blank control group with a growth rate that did not increase significantly. *T. obliquus* was cultured under 150 mg/L ciprofloxacin. In the first 72h, the algal cell density exceeded that of the blank group, indicating that a low concentration of ciprofloxacin hydrochloride promoted the growth of *T. obliquus* in a short time. After 96h, in the experimental group with a lower concentration, the growth rate of algal cells gradually returned to a normal rate. The algal density of *C. vulgaris* and *M. aeruginosa* at 336h was recovered in the control group but not in the highest concentration group. The growth of the algal cells was inhibited under the action of ciprofloxacin, the number of cells was reduced and even in the case of close to 100% inhibition, it still recovered after a while (Table 5).

Table 6 shows that the percentage of the algae density of *T. obliquus* at 96 h and 336 h in the lowest concentration (150 mg/L) and CG was 90 and 45%. The percentage in the highest concentration (600 mg/L) and CG was 50 and 29%. The results showed that population growth of *T. obliquus* was inhibited at a whole duration of 336h when it was treated with ciprofloxacin.

Table 6. Cell density of *T. obliquus* after treatment with different concentrations of ciprofloxacin hydrochloride.

Concentration(mg/L)	96 h	336 h	96 h	336 h
	Alage cell density (10^5 cells/mL)		Percentage of the CG	
Control group(CG)	18.601	56.497	-	-
150	16.698	25.693	90%	45%
300	14.033	22.977	75%	41%
450	12.694	20.703	68%	37%
600	9.2792	16.134	50%	29%

The percentage of the algae density of *S. quadricauda* at 96 h and 336 h in the lowest concentration (600 mg/L) group and CG was 48 and 42%. The percentage in the highest concentration (1500 mg/L) and CG was 25 and 13%. The results showed that the population growth of *S. quadricauda* was inhibited at a whole duration of 336h when it was treated with ciprofloxacin (Table 7).

Table 7. Cell density of *S. quadricauda* after treatment with different concentrations of ciprofloxacin hydrochloride.

Concentration(mg/L)	96 h	336 h	96 h	336 h
	Alage cell density (10^5 cells/mL)		Percentage of the CG	
Control group(CG)	32.750	139.038	-	-
600	15.825	57.900	48%	42%
900	9.426	39.718	29%	29%
1200	8.801	30.347	27%	22%
1500	8.344	18.533	25%	13%

As shown in Table 8, the percentage of the algae density of *M. aeruginosa* at 96 and 336 h in the lowest concentration (30 mg/L) group and CG was 76 and 100%. In the group treated with 30 and 45 mg/L of ciprofloxacin, the population density of *C. vulgaris* was restored to the population density level of the control group at 336h. The percentage of the algae density in the highest concentration (150 mg/L) group and CG was 12 and 26%. It was found that *M. aeruginosa* could detoxify after 8-day exposure to ciprofloxacin.

Table 8. Cell density of *M. aeruginosa* after treatment with different concentrations of ciprofloxacin hydrochloride.

Concentration(mg/L)	96 h	336 h	96 h	336 h
	Alage cell density (10^5 cells/mL)		Percentage of the CG	
Control group(CG)	395.981	728.532	-	-
30	301.566	727.692	76%	100%
45	252.332	727.728	64%	100%
60	122.416	722.140	31%	99%
150	45.971	191.921	12%	26%

The percentage of the algae density of *C. vulgaris* at 96 and 336 h in the lowest concentration (30g/L) and CG was 91 and 102%. In the group after being treated with 30 mg/L of ciprofloxacin, the population density of *C. vulgaris* was restored above the population density level of the control group at 336 h. The percentage of the algae density in the highest concentration (150 mg/L) group and CG was 11 and 18%. Thus, it was found that *C. vulgaris* detoxified after 11-day exposure to ciprofloxacin hydrochloride.

Table 9. Cell density of *C. vulgaris* after treatment with different concentrations of ciprofloxacin hydrochloride.

Concentration(mg/L)	96 h	336 h	96 h	336 h
	Alage cell density (10^5 cells/mL)		Percentage of the CG	
Control group(CG)	213.474	381.691	-	-
30	195.092	388.599	91%	102%
45	151.744	376.801	71%	99%
60	109.233	348.523	51%	91%
150	24.478	70.319	11%	18%

3.2.3. Effect Dose Curve of Enrofloxacin on Algae

The effect-dose curves of enrofloxacin on the four algae were fitted according to the 96h inhibition rate as shown in Fig. 4, and the curve equations and coefficients of determination are shown in Table 10. The results showed that the toxicity of enrofloxacin on algae was non-linearly related to the concentration. As shown in Table 7, the IC50 of enrofloxacin on *C. vulgaris*, *T. obliquus*, *S. quadricauda* and *M. aeruginosa* were 22.58, 195.6, 88.8, and 56.1mg/L, respectively.

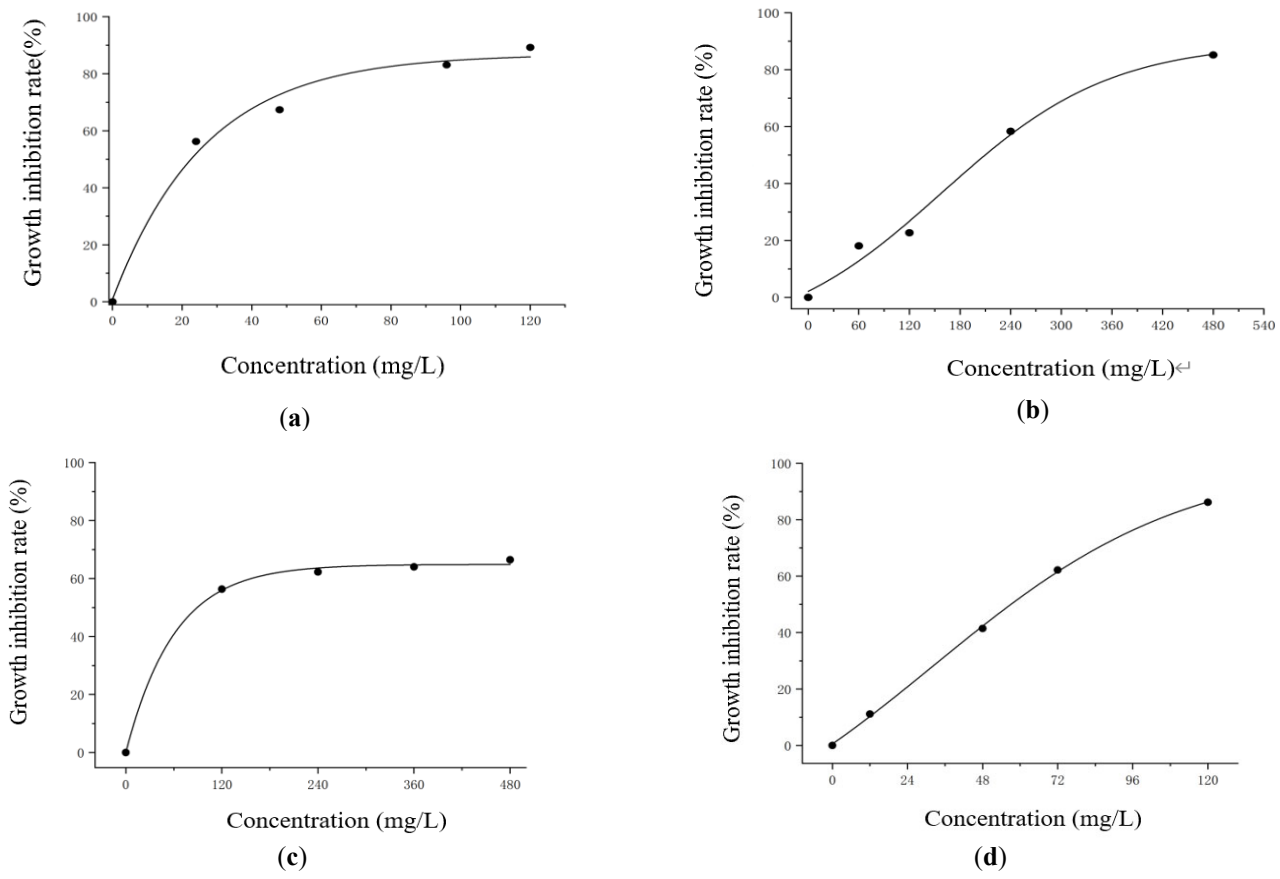


Fig. 4. (**a**) Inhibition curve of enrofloxacin on *C. vulgaris*; (**b**) Inhibition curve of enrofloxacin on *T. obliquus*; (**c**) Inhibition curve of enrofloxacin on *S. quadricauda*; (**d**)Inhibition curve of enrofloxacin on *M. aeruginosa*.

The growth of *C. vulgaris*, *T. obliquus*, and *M. aeruginosa* were almost completely inhibited when the enrofloxacin concentrations were 120 and 480 mg/L, and the inhibition rates were over 85%. *T. obliquus* showed the strongest tolerance to enrofloxacin, and that to *S. quadricauda*, the Scenedesmus family, was only 45%. *T. vulgaris* and *M. aeruginosa* had the least tolerance to enrofloxacin. However, with the increase of concentration, the slope of the curve gradually decreased and approached to 0, which indicated that the inhibitory effect of enrofloxacin on algae would not increase infinitely with an increase in concentration, but converge to a threshold value. After reaching this value, its inhibitory effect on algal cells would no longer increase regardless

of the concentration of enrofloxacin. As shown in the figures, the growth inhibition rate of *S. quadricauda* only increased by 9% when the concentration of enrofloxacin was increased from 120 to 480 mg/L. Its critical value was the smallest, which means that the maximum inhibition effect of enrofloxacin was the smallest.

Table 10. Dose curve equation and determination coefficient of enrofloxacin effect on algae.

Algal Species	Regression Equation	R^2
Chlorella vulgaris	y = 86.83 + (−170168.33 − 86.83)/(1 + exp((x+201.70)/26.57))	0.988
Tetradesmus obliquus	y = 49.69 + (−8.66 − 49.69)/(1 + exp((x+158.92)/99.63))	0.989
Scenedesmus quadricauda	y = 75.65 + (−28094.68 − 75.65)/(1 + exp((x-244.01)/41.23))	0.999
Microcystis aeruginosa	y = 110.35+ (−104.96 − 110.35)/(1 + exp((x-2.88)/56.47))	0.998

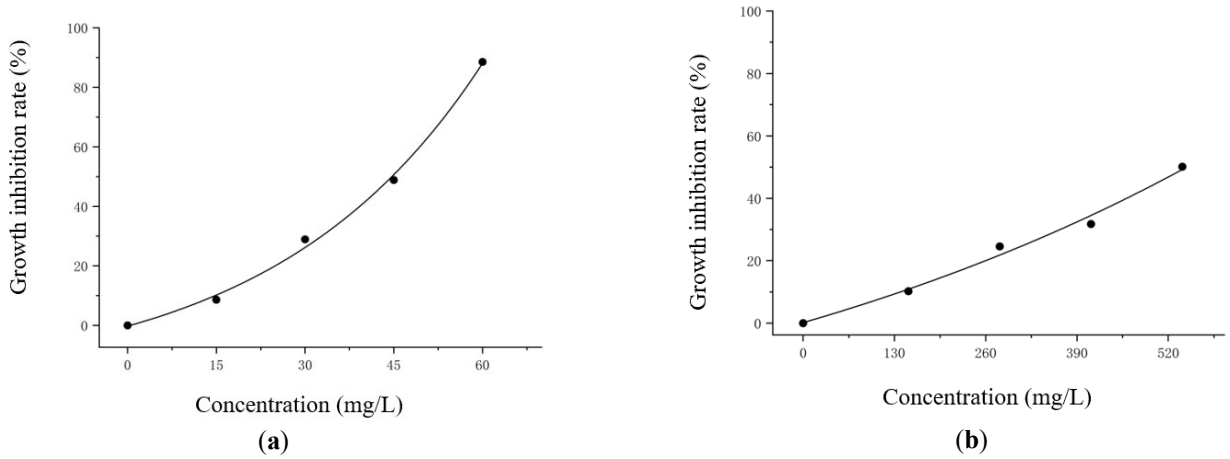
Table 11. 96h-IC50 values of two antibiotics to four kinds of algae

Algae Species / 96h-IC50 (mg/L)	*Chlorella vulgaris*	*Tetradesmus obliquus*	*Scenedesmus quadricauda*	*Microcystis aeruginosa*
enrofloxacin	22.58	195.6	88.8	56.1
ciprofloxacin hydrochloride	44.68	546.3	588.6	49.8

3.2.4. Effect Dose Curve of Ciprofloxacin Hydrochloride on Four Species of Algae

The effect-dose curves of ciprofloxacin hydrochloride on the four algae were fitted according to the 96h inhibition rate as shown in Fig. 5, and the curve equations and coefficients of determination are shown in Table 12. The toxicity of enrofloxacin on algae was non-linearly related to the concentration. As shown in Table 11, the IC50 of ciprofloxacin hydrochloride on *C. vulgaris*, *T. obliquus*, *S. quadricauda*, and *M. aeruginosa* were 44.68, 546.3, 588.6, and 49.8 mg/L, respectively.

All three green algae belong to *C. vulgaris*, but their tolerances to ciprofloxacin hydrochloride are different. *T. obliquus* and *S. quadricauda* had 12 and 13 times higher tolerance to ciprofloxacin hydrochloride than *C. vulgaris* while *M. aeruginosa* of cyanophyta had similar tolerance to *C. vulgaris*. Enrofloxacin was more toxic to three green algae than ciprofloxacin, but it was opposite to *M. aeruginosa*. The growth of *C. vulgaris*, *S. quadricauda*, and *M. aeruginosa* was almost completely inhibited when the ciprofloxacin hydrochloride concentration was 60, 900, and 150 mg/L. This indicates that the inhibitory effect of ciprofloxacin hydrochloride on algae did not increase infinitely with the concentration, but converges to a certain critical value. After reaching this value, no matter how the concentration of enrofloxacin increases, its inhibitory effect on algal cells no longer increases. The growth inhibition rate of *S. quadricauda* increased by 3% when the concentration of ciprofloxacin hydrochloride was increased from 900 to 1500 mg/L, which was the smallest critical value. This was the maximum inhibition effect of ciprofloxacin hydrochloride.



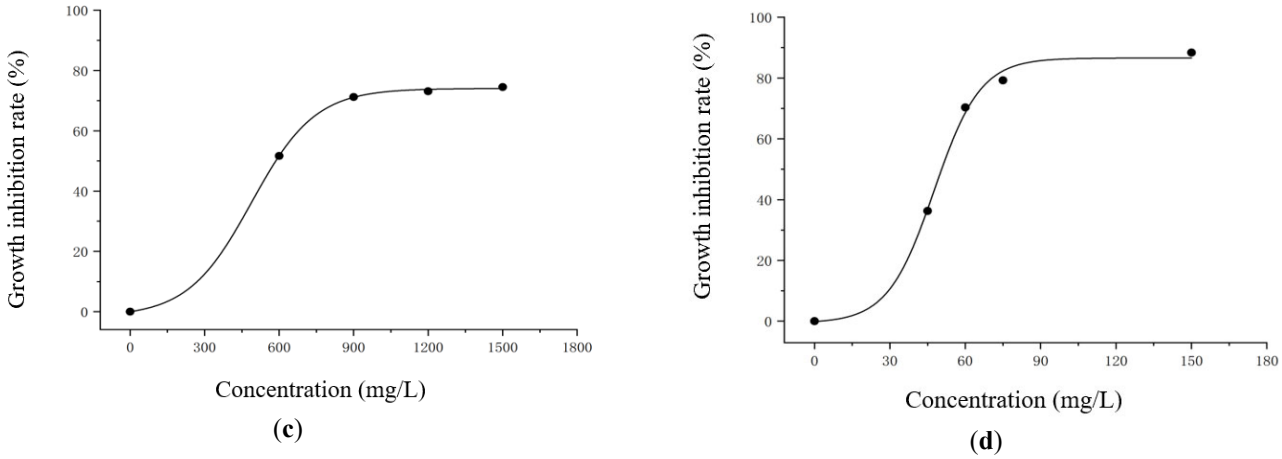


Fig. 5. (**a**) Inhibition curve of ciprofloxacin hydrochloride on *C. vulgaris*; (**b**) Inhibition curve of ciprofloxacin hydrochloride on *T. obliquus*; (**c**) Inhibition curve of ciprofloxacin hydrochloride on *S.quadricauda*; (**d**)Inhibition curve of ciprofloxacin hydrochloride on *M. aeruginosa*.

Table 12. Dose curve equation and determination coefficient of ciprofloxacin hydrochloride effect on algae.

Algal species	Regression equation	R^2
Chlorella vulgaris	y = 86.83 + (-170168.33-86.83)/(1 + exp((x+201.70)/26.57))	0.988
Tetradesmus obliquus	y = 49.69 + (-8.66-49.69)/(1 + exp((x+158.92)/99.63))	0.989
Scenedesmus quadricauda	y = 75.65 + (-28094.68-75.65)/(1 + exp((x-244.01)/41.23))	0.999
Microcystis aeruginosa	y = 110.35+ (-104.96-110.35)/(1 + exp((x-2.88)/56.47))	0.998

4. Discussion

4.1. Effects of Antibiotics on Phytoplankton

Kim reported the toxic mechanism of three antibiotics (tetracycline, lincomycin, and sulfamethazine) on Crescent algae. the growth and metabolism of Crescent algae changed mostly with the concentration change of antibiotics, and the sensitivity of Crescent algae to antibiotics was stronger than that of other organisms such as *Daphnia magna* and *Vibrio freundii* [28]. Andreozzi found that lincomycin had no effect on *Pseudomonas aeruginosa*, but significantly affected the growth of cyanobacteria [29]. Liu found that erythromycin, ciprofloxacin, and sulfamethoxazole had obvious inhibitory effects on the photosynthesis of *Cunninghamia lanceolata* [30].

4.2. Effects of Quinolone Antibiotics on Planktonic Microalgae

It has been reported that the effect of enrofloxacin on *T. obliquus* increases with the exposure concentration increase [31], and its growth inhibitory effect is also continuously enhanced. Su found that after 96 hours of enrofloxacin exposure, the content of soluble protein in *C.vulgaris* gradually decreased with the increase of enrofloxacin concentration. This indicated that cell viability decreased with the increase in drug concentration [32]. While SOD and MDA had a negative correlation with drug concentration, the active oxygen in the organism increased with the increase in drug concentration. The algae cell damage degree increased, that is, the damage degree of the algae cell membrane system was deepened. The results of this research were consistent with those of previous studies.

Wan's study of the three antibiotics, levofloxacin, ofloxacin, and erythromycin, showed "low promotion and high inhibition" on the growth of two microalgal species [33]. Shi's research also showed that low-concentration groups of three quinolone antibiotics (pipemidic acid, ciprofloxacin, and norfloxacin) could promote the growth of *Chlorella pyrenoidosa* [34]. Under low concentrations of antibiotics, the antioxidant system of green algae did not change significantly. Under high concentrations of antibiotics, the activity of green algae antioxidant enzymes was induced, the content of malondialdehyde increased significantly, and the growth of algae was inhibited, which was consistent with the results of this experiment. The results of Gao'sresearch on three antibiotics (tetracycline, sulfadiazine, and sulfamethoxazole) to *M. aeruginosa* showed that the longer the time, the more obvious the inhibitory effect [35]. The inhibitory effect of antibiotics on microalgae changed with time, which was determined by the type of antibiotics.

Nie reported that the 96h-IC50 of ciprofloxacin on *C. vulgaris* was 20.61 mg/L [36]. The IC50 in this research was 44.68 mg/L. Wang reported that the 96h-IC50 of enrofloxacin on *C. vulgaris* was 0.1245 mg/L [37], while the IC50 in this research was 22.58 mg/L. Chen reported that the 96h-IC50 values of enrofloxacin and ciprofloxacin for *T. obliquus* were 59.44 and 51.71 mg/L [38], and the IC50 in this research was 195.6 and 546.3 mg/L. Yang found that the 96h-IC50 of enrofloxacin on *M. aeruginosa* was 0.0846 mg/L [39], and the IC50 in this research was 56.1 mg/L. The difference in the 96h-IC50 of enrofloxacin and ciprofloxacin on each species in this research and other reports may be due to the different test temperatures (25–34°C) in experiments or due to the better resistance of the algal species to the toxic effects of antibiotics after multiple generations of laboratory culture.

The results of this research showed that *C. vulgaris* and *M. aeruginosa* had similar sensitivity to the two quinolone antibiotics, while *T. obliqua* and *S.quadricauda* showed similar sensitivity. From the point of view of the morphological characteristics of cells, *C. vulgaris* and *M. aeruginosa* are quasi-spherical, while *S.quadricauda* and *T. obliquus* are ovoid, so it is worth further exploring whether the tolerance of microalgae to antibiotics is related to their morphological structure.

The four curves in Fig. 4 leveled off after the concentration reached a certain value, and the inhibition rate no longer changed significantly with the increase in concentration. For example, the growth inhibition rate increased by 9% from 120 to 480 mg/L of enrofloxacin. The curves in Figs. 5(c) and (d) showed a clear "S" shape. The growth inhibition effect of enrofloxacin and ciprofloxacin did not change significantly from a certain concentration even though the concentration increased over the concentration. The toxicological effects of two quinolone antibiotics, enrofloxacin and ciprofloxacin hydrochloride on *Lentinus subcentricus* were investigated by Lian. He showed that the higher the concentration of the antibiotics, the more pronounced the inhibition of growth on chlorophyll and extracellular polymer. However, when the concentration reached a certain value, the inhibition reached its threshold [40], which coincides with the results of this research.

5. Conclusions

The effects of enrofloxacin and ciprofloxacin hydrochloride on the four microalgae species showed that the growth inhibition rate increased with increasing concentrations of the antibiotics, and there was a good dose-effect relationship between the two antibiotic concentrations and the growth inhibition rate of microalgal cells. However, enrofloxacin and ciprofloxacin had their inhibition threshold for the microalgae concentration. Among the four microalgae, the inhibition threshold of these two quinolone antibiotics was the lowest in *S.quadricauda*. The IC50 values of enrofloxacin on *C. vulgaris*, *T. obliquus*, *S.quadricauda*, and *M. aeruginosa* were 22.58, 195.6, 88.8, and 56.1 mg/L, respectively. The IC50 values of ciprofloxacin on *C. vulgaris*, *T. obliquus*, *S. tetragoni*, and *M. aeruginosa* were 44.68, 546.3, 588.6, and 49.8 mg/L, respectively. Enrofloxacin was about 2, 2.8, and 6.6 times more toxic than ciprofloxacin hydrochloride for *C. vulgaris*, *T. obliquus*, and *S.quadricauda*, respectively, while ciprofloxacin hydrochloride was slightly more toxic than enrofloxacin for *M. aeruginosa*. Enrofloxacin was more toxic for the three green algae, while ciprofloxacin hydrochloride was more toxic for *M. aeruginosa*. The susceptibility of the four algae to enrofloxacin was large in the order of *T. obliquus* < *S.quadricauda* < *M. aeruginosa* < *C. vulgaris.* The order of the susceptibility of the four algae to ciprofloxacin was *S.quadricauda* < *T. obliquus* < *M. aeruginosa* < *C. vulgaris*. For these two quinolone antibiotics, *Scenedesmus* was the least sensitive and most tolerant, while *C. vulgaris* was the most sensitive.

The microalgae was damaged or even completely inhibited in the first 96 h when the concentration of these two quinolone antibiotics was high. That is, the density of microalgae increased slowly in the first 96 h. Although the algae density began to increase gradually after 96 hs, the inhibitory effect of enrofloxacin on the four algae increased with time compared with the control group. The inhibitory effect of ciprofloxacin hydrochloride on *T. obliquus* and *S.quadricauda* increased, while the inhibitory effect on *C. vulgaris* and *M. aeruginosa* decreased. Increased toxicity gradually increases the algae density, which indicates that the microalgae had a strong ability to repair the damage and regenerate themselves in quinolone antibiotics. Under the same inhibitory conditions, except *M. aeruginosa*, the recovery degree of three green algae species with ciprofloxacin hydrochloride was higher than with enrofloxacin.

Low concentrations of enrofloxacin (< 36 mg/L) could inhibit the growth of *M. aeruginosa* in the first 4 days, but provoked its population growth after 240 h. Then, the algae density exceeded that of CG and reached more than 10^7 cells/mL. When the concentration of ciprofloxacin hydrochloride was less than 150 mg/L, the density of *M. aeruginosa* at 336 h was almost the same as that of the control group, but not higher than that of the control group. This indicates that long-term exposure to low concentrations of enrofloxacin may be more prone to the outbreak of *M. aeruginosa* blooms.

Author Contributions: Conceptualization, Qin-Ming Xie, Ta-Jen Chu and Yu Xia; methodology, Qin-Ming Xie; software, Yu Xia; validation, Qin-Ming Xie, Ta-Jen Chu and Yu Xia; formal analysis, Qin-Ming Xie, Ta-Jen Chu and Yu Xia; investigation, Qin-Ming Xie; resources, Qin-Ming Xie; data curation, Qin-Ming Xie and Yu Xia; writing—original draft preparation, Qin-Ming Xie, Ta-Jen Chu and Yu Xia; writing—review and editing, Ta-Jen Chu; visualization, Qin-Ming Xie and Ta-Jen Chu; supervision, Qin-Ming Xie. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by Fujian Spark Program (No. 2019S0039),Fujian Spark Program (No.2019S0026), Provincial Natural Science Foundation of Fujian Province (No. 2013J01136) and Fujian Provincial Key Science, Technology Guidance Project (No. 2021N0013),and Jimei University grant (No. C619061).The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgments: We thank Shu-Hong Wang and Yi-Jia Shi for their contributions to the suggested revision comments to manuscript. Useful suggestions from anonymous reviewers were incorporated into the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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