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The enhancement by the artificial controlled culture for the algal treatment of antibiotic ceftazidime: a three-step responses performance and high removal efficiency

Yingxiang Du[1,2], Yunqing Feng [1], Ruixin Guo[1,*], Jianqiu Chen[1,**]

1. China Pharmaceutical University, Key laboratory of Drug Quality Control and Pharmacovigilance, Nanjing 210009, China

2. State Key Laboratory of Natural Medicines, Nanjing 210009, China

*Corresponding author:

Ruixin Guo: China Pharmaceutical University, Key laboratory of Drug Quality Control and Pharmacovigilance, Nanjing 210009, China, Tel.: + 86 25 86185190, Fax: + 86 25 86185190. E-mail address: ruixinguocpu@163.com

**Corresponding author:

Jianqiu Chen: China Pharmaceutical University, Key laboratory of Drug Quality Control and Pharmacovigilance, Nanjing 210009, China, Tel.: + 86 25 86185190, Fax: + 86 25 86185190. E-mail address: cjqalga@163.com

Abstract

The improved activity of the alga is critical in the biological enhanced treatment to remove contamination. Organisms usually perform compensatory response resulting from an unfavorable condition. This work investigated the effect of the artificial controlled culture before the treatment, which provoked the possible algal compensatory response and helped the algae to perform a better removal capability on the antibiotic ceftazidime in the subsequent treatment process. The removal efficiency could be improved up to 99.15% in 6 h when the algae was under the artificial controlled light conditions before the treatment process. Additionally, higher removal efficiency (98.57% and 99.98%) was obtained after the artificial control on N and P, respectively. It suggests that the algae displayed a sequence of response during the exposure to the antibiotic after the artificial controlled culture in three steps: compensatory response, adsorption-consumption acceleration and acclimation. It might be the first time that the artificial conditions changing were controlled to improve the removal efficiency. Our work pointed out a new method for biological enhancement technologies in the antibiotic wastewater treatment.

Keywords: ceftazidime; green alga; artificial conditions changing; removal efficiency; biological enhancement

Introduction

Nowadays chemical pollution from heavy metals, solvents, dyes and pesticides, is one of the major threats to the environment safety and human health [1]. These pollutions could reach the environment through several different ways, such as hospital effluents, industrial wastewater and excrement from humans or livestock [2, 3]. Antibiotic pollutions become a serious and urgent issue because of their high consumption rate [4]. Antibiotics are widely applied as therapeutics and growth additives in livestock and aquaculture industries [5]. Although the concentration of the residue antibiotic in the aquatic and terrestrial environment is generally not high (μg/L or μg/kg, respectively) [6], they are considered to be emerging pollutants because of their immediate and potential toxic effects. Several studies have shown the toxic effects of some antibiotics on the aquatic species, such as monocotyledonous macrophyte *Lemna minor* and freshwater crustacean *Daphnia magna* [7, 8]. Moreover, low concentrations of antibiotics in the environment may also result in the emergence of antibiotic resistant bacteria and antibiotic resistance genes in a long time, which have serious impacts on the ecosystem [9-11].

To date, different elimination techniques have widely been applied in municipal wastewater treatment plants (WWTPs), including the conventional techniques and the advanced methods [6, 12, 13]. Biological treatment such as the activated sludge is a widely used technology in current WWTPs, while the removal efficiency of antibiotics is still unsatisfactory. Therefore WWTPs have become one of the dominant pollution sources for antibiotics [14]. Thus, it is necessary to search for more efficient,

safe, practical and economical treatment approaches.

Microalgae are autotrophic primary producers in maintaining the ecological balance. Many studies have shown that microalgae have the capability to accumulate and remove environmental contaminations, such as heavy metals, insecticides and other organic chemicals [15-18]. There is also a good application of the green alga *Chlorella vulgaris* on the removal of the antibiotic tetracycline in the algal ponds [19]. Environmental factors often play an important role in the removal process of the microalgae. The light and nutrient conditions have a strong influence on the fate of contaminants [20-22]. Inorganic additives such as N and P elements were used to increase the cell number as well as the activities to degrade the contamination [23, 24]. And more importantly, alga could tolerate constantly the environmental impacts and adapt to the new conditions, such as the changes in temperature, humidity, salinity, irradiance and nutrient availability [25]. In addition, organisms usually perform compensatory response resulting from the restricted food availability or the unfavorable environmental condition [26]. For example, the compensatory growth of the marine alga *Phaeodactylum tricornutum* was provoked by darkness [27]. Other previous study also indicated that the algal cells were able to acclimate rapidly to current light levels and resume growth fast during the recovery period in the light [28].

Thus, the stimulated activity, like growth capacity or photosynthesis, as of the algae is critical in the biological enhanced treatment to remove contamination. Although many previous studies revealed how the light and nutrition condition influenced the capability of the algae to remove compounds, we are especially interested in whether

the artificial controlled cultures before the treatment provokes the possible algal compensatory response and helps the algae to perform a better removal capability on the antibiotic in the subsequent treatment process. In this work, the removal performance of the target antibiotic ceftazidime by the green alga *C. pyrenoidosa* in the algal reactor was evaluated and compared. The antibiotic ceftazidime, used in the present study, is a third-generation semi-synthetic cephalosporin and has a strong effect against a wide variety of gram-negative bacteria [29]. For widely consumption and the limit of the conventional wastewater treatment technique, ceftazidime and other kinds of cephalosporin were detected in wastewater and river water samples [30]. Our previous research has also compared the removal efficiency of the antibiotic by an activated sludge treatment and an algal treatment [31]. The results indicated that the green algae *C. pyrenoidosa* performed a satisfactory growth ability under the impact of ceftazidime. Thus, the aim of this work is to explore the effect of the artificial controlled culture before the treatment on the removal capacity of the green algae on ceftazidime in the treatment process. We provide a hypothesis that the artificial controlled culture before the treatment could meaningfully improve removal capability of the algae on the antibiotic in the subsequent treatment process. It might be a new method for the biological enhancement in the antibiotic wastewater treatment.

Materials and methods

Chemicals

The antibiotic ceftazidime (purity >98%) used in the tests was purchased from Yabang

investment holding group CO., LTD. Methanol and acetonitrile were HPLC grade. Other chemicals and reagents were analytical grade.

Algal cultures

The green algae *C. pyrenoidosa* (FACHB-1220) which purchased from the Wuhan Hydrobiology Institute of Chinese Academy of Sciences, was pre-cultured in BG-11 media at 25 ± 1°C and 4000 lux illumination (40 μmol photons m^{-2} s^{-1}) with a light: dark interval of 12 h: 12 h. The algae was cultured for normally to reach the logarithmic growth phase in 3 d and prepared for the subsequent experiments. The experiment had three replications per treatment.

Ceftazidime analysis

Ceftazidime was analyzed using a high-performance liquid chromatography (LC-10A, Shimadzu) apparatus coupled with UV detector. Stock solutions (1 g/L) of ceftazidime were dissolved in BG-11 media. The working solutions (40 mg/L) used in the treatment were then diluted from stock solutions. Antibiotic samples were separated and determined with an Inertsil ODS column (4.6 mm × 150 mm, 5 μm). The mobile phase of ceftazidime was acetonitrile-pH 3.9 phosphate buffer (volume ratio was 7:93). The flow rate was 1.0 mL/min with 20 ± 1.0 °C. The wavelength of the UV detector was 255 nm. Quantitation was performed using external standards and was based on peak areas.

Experimental set-up

In the preliminary experiment, the removal rates of the target antibiotic tceftazidime by a dead and living algal cells were evaluated. We also evaluated the concentration

of the antibiotic without the green alga to determine the self-degradation rate. Additionally, the green algal cells was mixed with the antibiotic in 6 h and then washed by the distilled water several times. The concentration of the target antibiotic in the washed water and in the algal cells was also detected to reveal whether the cell surface and intracellular sorption occurred. The present study was performed in three phases according to different controlled conditions. All the parameters of the artificial controlled culture before the treatment was present in Table 1, Table 2 and Table 3 for details. The initial algal density and the concentration of ceftazidime in all phases were 11.00×10^6 cells/mL and 40.00 mg/L, respectively. The total process in every phase included the algal culture process before the treatment (72 h) and the antibiotic treatment process (6 h). The removal rate was measured at 0.5, 1, 2, 3 and 6 h during the treatment process. In phase I, the artificial controlled culture before the treatment was performed in different light-control conditions, but in the normal nutrition level (seen in Table 1). To evaluate the effect of the light-control culture before the treatment, three groups of *C. pyrenoidosa* were cultured in dark for 24, 48 and 72 h, respectively. Then, the green algae in the three groups treated ceftazidime at 40.00 mg/L under the light condition, respectively (Group 1, 2 and 3). The effect of the artificial light-control condition in the antibiotic treatment process should also be taken into account. Thus, the algal cells, which were cultured under the normal light condition before the treatment were then treated ceftazidime under the dark and light condition, respectively (Group 4 and 5). The illumination intensity was confirmed uniformly as 4000 lux during the whole process. Additionally, the artificial

nutrition-control in the culture before the treatment was considered in the following two phases (see in Table 2 and 3): In Phase II, the artificial controlled cultures before the treatment were carried out under different nitrogen levels. In Group 6, *C. pyrenoidosa* was cultured under the nitrogen starvation condition in 72 h and treated the target antibiotic under a nitrogen starvation condition. The algal cells of another three groups were cultured under a nitrogen-lack condition for 24, 48 and 72 h, respectively (Group 7, 8 and 9). Then, the algal cells in the three groups were exposed to ceftazidime at a normal nitrogen concentration (BG-11 media, N: 1.50 g/L), respectively. In Group 10, we also assessed the removal capacity on ceftazidime when the green alga was cultured in an artificial nutrient starvation condition before the treatment and then treated the antibiotic under an artificial double nutrient level of the normal BG-11 media (N: 3.00 g/L). In phase III, the artificial controlled cultures before the treatment were conducted under different phosphorus levels, as similar as described in Phase II (see in Table 3, Group 11-15). The light condition in Phase II and III was confirmed as 4000 lux in illumination and 12:12 (L: D) in photoperiod. $NaNO_3$ and K_2HPO_4 was used as the N-resource and P-resource, respectively in the artificial nutrition-control. All treatments were conducted in an algal reactor (5 L glass cylinder, more details have been present in Fig.1). Magnetic stirrers inside the reactor were used to maintain the suspension of the algal cells. The algal population density was determined at the same time intervals as described above. The rate of population increase (r) was calculated according to the formula: $r=1/t_n$ ($\ln N_n - \ln N_0$); where N_n is the algal cell density at time t_n, N_0 is the initial algal cell density, and t_n is time for the

final measurement after the beginning of the experiment [32]. Samples at time interval were centrifuged at 4800 rpm for 15 min. Following the filtration of supernatant through 0.45 μm glass fiber filters, the residual ceftazidime concentration was quantified by HPLC. The chlorophyll a of the algal cells were extracted by 95% methanol [33] and the content of the photosynthetic pigments was calculated following the formula described [34]. The algal cell size was determined through Laser diffraction phaseicle size analyzer (Shimazu, SALD-2201). The concentration of ceftazidime without the alga was measured to evaluate the self-degradation of the antibiotic.

Statistical methods

The mean and standard deviation of three replicates of the treatment was calculated. The results were analyzed with SigmaPlot (Version 12.0) and SPSS (Version 11.5). The statistical analysis of the data between different treatment groups was carried out using one-way analysis of variance (ANOVA). If the ANOVA result was significant at the 0.05 confidence limit, the least significant difference (LSD) test was utilized to find out where the difference occurred.

Results

The effect of the artificial controlled culture on the algal growth during treatment

The rates of the algal population increase varied with the exposure time after the green alga *C. pyrenoidosa* underwent different artificial controlled cultures (see in Fig.2). In the whole treatments, an apparent decrease in growth was observed during the different time periods. After a 24, 48 and 72 h of dark, respectively, the algae grew better than that under the normal light condition. However, it was worth noting that

the growth situation of the algae was worst under the dark treatment condition. In addition, with respect to the N-limit and the N- lack condition, it had different impacts on the algal growth (see in Fig.2B). At the first 1 h, the alga under the N-lack condition grew faster than that under all of the N-limit conditions. However, the given artificial controlled culture of N-limit condition stimulated the growth of the green algae during 1 to 3 h significantly (Group 7). Different from nitrogen, the effect of phosphorus on the rate of the algal population increase was obvious. Especially, the artificial controlled culture of a 72 h P-starvation significantly stimulated the growth of the alga (Group 9).

The effect of the artificial controlled culture on the removal of ceftazidime

In the preliminary experiment, the removal rates of ceftazidime by the dead and living algal cells were evaluated and compared. It indicated that the living algae had a better removal capacity than that of the dead one. Moreover, the self-degradation rate of the antibiotic was about 15% after 48 h, while the sorption rate of the living algal cells on the antibiotic was only 0.91%. Considering that algal population density also changes during the removal process, the removal rate of the unit algal density per hour, i.e., the "cellular removal rate", should better reflect the removal capacity at any given time. Fig.3 showed the average removal rates of ceftazidime by the unit algal density when the algae was under the different artificial light, N or P controlled culture before the treatment, respectively. The highest removal efficiency of the unit algal cell in the different artificial light and nutrient controlled conditions was obtained during 1-3 h. Compared with the group under the normal light and nutrition condition, the average

removal rate of the unit algal cell increased under the artificial light-limit condition while decreased under the artificial N or P-limit culture. Meanwhile, it was noteworthy that the average removal rates of ceftazidime significantly decreased when the algae under the artificial dark treatment, N-starvation for 72 h and P-starvation for 72 h before the treatment. Additionally, with respect to the total removal rate of 6 h, if the green algae underwent a 48 h artificial P-starvation before the treatment and an artificial P-abundant condition during the treatment (Group 15), the highest removal efficiency (99.98%) could be obtained.

The effect of the artificial controlled culture on the algal physiological status

The change of chlorophyll a content was also observed during the treatment process when the green algae underwent the different artificial controlled conditions (see in Fig.4). Our result indicated that the rates of the algal chlorophyll a content increase were negative at first, while were positive during the later periods of the treatment under the artificial light and N-controlled conditions. During 3 to 6 h, the rates of chlorophyll a content increase were significantly higher than that under the normal condition when the green algae underwent a 72 h dark culture, a 48 h N-starvation with an abundant nutrient recovery, and a 48 h P-starvation with an abundant nutrient recovery. However, the change of chlorophyll a content varied with the experienced length of the algae under the artificial N or P-starvation culture before the treatment. As shown in the figure, when the algae underwent a 48 h artificial N-starvation culture (Group 8), the chlorophyll a content was accumulated during 3 to 6 h, while it decreased when the algae was under a 48 h P-starvation culture (Group 13). The

average cell size changed when the algae treated ceftazidime for 6 h under the different artificial controlled conditions (see in Fig.5). The cell size significantly increased 6.3% ($p < 0.05$), 7.8% ($p < 0.01$), 7.3% ($p < 0.01$) when the algae underwent a 24, 48 and 72 h dark culture before the treatment, respectively. In addition, the artificial N or P starvation before the treatment and in the treatment process also influenced the algal cell size significantly. A declined cell size ($p < 0.01$) was observed in the artificial N-lack culture (Group 6), the artificial P-lack culture (Group 11) and the artificial P-limit cultures (Group 12). However, the change rates of the cell size arrived up to 21% ($p < 0.01$) and 17% ($p < 0.01$) when the algae treated the antibiotic at an abundant nutrient recovery after an artificial 72 h N-starvation and P-starvation culture, respectively.

Discussion

The present study evaluated the removal efficiency when the algae was under the artificial culture at varied light-controlled conditions before the treatment. It should be noted that all removal rates in previous studies were reported for the algal population as a whole. Considering that algal population density also changes during the removal process, the enhanced total removal efficiency might be attributed to the increasing algal biomass. Our result also indicated that the algal population increased during the treatment (Fig.2). Thus, to better reveal whether the algal removal capacity was improved by the artificial controlled culture, the average removal rates by the unit cell was reported at any given time. Additionally, the total removal efficiency was also considered to better compare with the previous reported results. In Fig.3, it showed

that under the light controlled condition treatment process, due to the same self-degradation of the antibiotic occurrence, the average removal rates among the five groups were different depending mainly on the different artificial light-limit cultures before the treatment. Several previous studies also obtained the similar results. For example, nearly 70% of aniline was degraded after 4 h of irradiation, while no obvious bio-degradation of the compound was observed in the dark [15]. Similarly, the removal of 2, 4-dichlorophenol (DCP) and bisphenol A (BPA) by *Chlorella fusca* was based on light [35, 36]. The capability of the algae to remove compounds may be highly dependent on light, which implies that the removal of organic contaminations by algae was closely linked with photosynthesis [37]. In our present study, the green alga *C. pyrenoidosa* was cultured in the artificial light-limit condition and the artificial dark condition before the treatment, respectively. After the green algae was transferred to the subsequent treatment in light condition, the removal efficiency of ceftazidime was obviously improved. It produced the evidence that not only the light itself, but also the changed light condition played a crucial role to remove the antibiotic in the treatment process.

Nutrition control is another factor which should be considered. Previous studies showed that an impact at a relevant low dose activated an adaptive response of organisms to resist to different degree of nutrition limitation [38]. In the present study, the artificial N-limit condition before the treatment also could improve the final removal capacity of the green algae. The relevant higher removal rate of ceftazidime was observed at 1-3 h when the algae underwent a 24 and 48 h artificial N-starvation

culture, respectively. However, although the rate of population increase was higher when the green algae was transferred to an N-abundant condition for treatment after the 48 h artificial N-starvation culture (Fig.2B), while the removal capacity was not improved significantly (Fig.3B). It suggests that the nitrogen in the media could be easier utilized by the green algae than the target antibiotic. A possible competitive assimilation caused the relevant lower removal rate. In addition, the green algae performed better removal capacity when it was under most of the artificial P-limit cultures before the treatment, while it was more susceptible to the P-lack condition.

The removal rate by the unit cell in three treatment periods was only 1.51%, 3.77% and 0.79%, respectively, when the algae underwent a 72 h artificial P-starvation culture (Group 14). Although the algal population increased continuously during the treatment process, the total removal rate at the end of 6 h was only 89.26%, which suggests that the P-limit might be under a given threshold. The artificial controlled condition could be harmful for the activity of the green algae if the condition was lasting longer than that the algae endured. We also observed a declined content of chlorophyll *a* at the P-limit conditions. In the present study, if the algae underwent an artificial N-starvation culture before the treatment, the removal capacity of the unit algae cell was enhanced in the three periods of the treatment process. It indicated a possible compensatory response occurred when the green algae underwent an artificial nutrition-limit condition and treated the antibiotic under a normal nutritional condition. The nutrition-lack group in the present work indicated that the algae underwent a nutrition-lack culture before the treatment and also under a nutrition

-lack condition in the treatment process (Group 6). Therefore, the removal rate of the N-lack group was lower than that in other N- limit groups. However, it's worth noting that the results of the P-lack group (Group 11) were not the same as that of the N-lack group, which indicated that the mechanism of P was different from that of N.

In addition, the concentration of the antibiotic in the algal cells was measured. The concentration of the intracellular ceftazidime was only 0.54% of the total antibiotic concentration added in the treatment, which indicated that the algal degradation might play the main role in the antibiotic removal. Thus, in the following study, we will consider how the antibiotic has been degraded to different fragments by HPLC-MS/MS, which could help us to better understand the removal processes by the green algae on the antibiotic. On the other hand, the nutrition conditions in the reactor have been controlled and considered. However, the limit extracellular N or P concentration does not mean the limit intracellular concentration. Therefore, how the intracellular nutrition concentration changed under the different extracellular nutrition conditions should be studied deeply. It could have a possible compensatory response of the green algae under an unfavorable extracellular condition. Details on the nutrition conditions should be considered in the following study such as the relationship of the extracellular and intracellular nutrition conditions, the form of N and P. It could be better to reveal the mechanism of the controlled culture to enhance the final removal efficiency of the green algae on the antibiotic.

The stimulated activity of the organism is critical in the biological enhanced treatment to remove contamination. Usually, the inability of conventional biological

technologies to effectively remove the hazardous substances claims for enhancement treatment technologies like low-intensity ultrasound [39] or magnetic field [40], while the relevant applications in algae were limited. Many previous studies have focused the effects of nutrient limitation on the physiology and metabolism of microalgae, such as the substance accumulation [41] and cell composition [42] [43, 44]. And, more remarkable, once the environmental condition changed, the algal physiological response in the photosynthetic performance, matter metabolism, regulatory functions and cellular signaling could help the algae adapt to new conditions [45, 46]. Compared with the results in Fig. 2 and Fig.3, it indicated that the algae performed a relative better growth capacity at the first 1 h (0-1 h) after the condition changing, while the best removal capacity of the alga on the antibiotic was performed in the next period (1-3 h). Thus, our study implied that the artificial controlled culture before the treatment could provoke a possible algal compensatory response and help the algae to perform a better removal capability on the antibiotic in the subsequent treatment process. A possible model postulates that the green algae displayed a sequence of response during the exposure to the antibiotic after the artificial controlled culture in three steps: In step I, the algae received the stimulation of the changing conditions and released a possible compensatory response. In step II, the algae accelerated the adsorption and the consumption of the antibiotic, and the acclimation in step III (see in Fig.6). Due to the low operating costs and high efficiency, microalgae have been employed to remove various organic and inorganic materials from wastewater. For antibiotic removal, 36.9% of norfloxacin were removed by the green algae *C. vulgaris* [47], a reduction of

44% of the initial concentration of sulfonamides was attributed to the uptake from the algae *Ulva lactuca* [48]. Although about 69% of tetracycline could be removed, the treatment time was needed to extend to 62 days [49]. The removal rate of the target antibiotic in the present study was always higher than the above reported results. In additional, compared with the relevant normal light and conditions (Group 5), the removal efficiency could be improved up to 99.15% in the 6 h algal treatment when the algae was under the artificial controlled light conditions before the treatment. Additionally, higher removal efficiency (98.57% and 99.98%) was obtained after the artificial control on N and P, respectively. It suggests that the removal efficiency could be improved further just by the artificial controlled conditions rather than the chemical enhanced process such as Fenton oxidation or O_3. Although most of the previous studies revealed how a given light or nutrient conditions influenced the removal of compounds by the algae, it might be the first time to change the artificial conditions between the culture process and the subsequent treatment process, which improved the final removal efficiency in the algal reactor. In addition, light-limit caused less light usage in the whole treatment process than before, which could save more energy and decline the treatment cost. It is worth noting that the algal cell size was correlated with nutrient, light or temperature, especially the xenobiotics impact [50-52]. In the present work, the algal cell size varied during the treatment might be viewed as a response to antibiotics such as the intracellular substance storage or consumption by the algal absorption and graduation of the xenobiotic compounds. Thus, it is necessary to reveal the activity of algae including the physiological

response like SOD, MAD and the algal mechanism pathway in further research, which might help us to better understand the removal process by the algae on antibiotics.

In conclusion, the artificial controlled culture before the treatment had a significant effect on the removal of ceftazidime in the algal reactor. After three different artificial cultures in the light-limit condition, the removal rates were all higher than 98% at the end of the treatment. Similarly, better removal capacity was observed when the green algae was under the artificial controlled culture of a 24 h or 48 h N-starvation, respectively. Moreover, the removal capacity of the algae was improved in most of the artificial P-limit culture except for the 72 h P-starvation. The present study indicated that the artificial controlled culture before the treatment could improve the algae for a better removal capability in the subsequent treatment process. Our work pointed out a new method for biological enhancement technologies in the antibiotic wastewater treatment.

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Table 1 The design of light-control

Group	Type of light-control	Algal culture process before treatment (h)			Antibiotic treatment process (h)
		0-24	24-48	48-72	0-6
1	Light limit in culture	N	N	D	L
2		N	D	D	L
3	Dark in culture	D	D	D	L
4	Dark in treatment	N	N	N	D
5	Light normal	N	N	N	L

N: in the normal photoperiod (Light: Dark = 12h: 12h)
L: in the light condition
D: in the dark condition

Table 2 The design of nutrient-control

Experiment group		Type of phosphorus control	Nitrogen control				Other ingredients level			
			Culture process before treatment (h)			Treatment process (h)	Culture process before treatment (h)			Treatment process (h)
			0-24	24-48	48-72	0-6	0-24	24-48	48-72	0-6
Part	**6**	N-lack	S	S	S	S	N	N	N	N
II	**7**	N-limit	N	N	S	N	N	N	N	N
	8	N-limit	N	S	S	N	N	N	N	N
	9	N-limit	S	S	S	N	N	N	N	N
	10	N-limit	N	S	S	D	N	N	N	D

S: in nutrient starvation

N: in normal concentration in BG-11 medium (N: 1.50 g/L; P: 0.04 g/L)

D: in double of the normal concentration in BG-11 medium (N: 3.00 g/L; P: 0.08 g/L)

Table 3 The design of Phosphorus-control

Experiment group		Type of phosphorus control	Phosphorus control				Other ingredients level			
			Culture process before treatment (h)			Treatment process (h)	Culture process before treatment (h)			Treatment process (h)
			0-24	24-48	48-72	0-6	0-24	24-48	48-72	0-6
Part	**11**	P-lack	S	S	S	S	N	N	N	N
III	**12**	P-limit	N	N	S	N	N	N	N	N
	13	P-limit	N	S	S	N	N	N	N	N
	14	P-limit	S	S	S	N	N	N	N	N
	15	P-limit	N	S	S	D	N	N	N	D

S: in nutrient starvation

N: in normal concentration in BG-11 medium (N: 1.50 g/L; P: 0.04 g/L)

D: in double of the normal concentration in BG-11 medium (N: 3.00 g/L; P: 0.08 g/L)

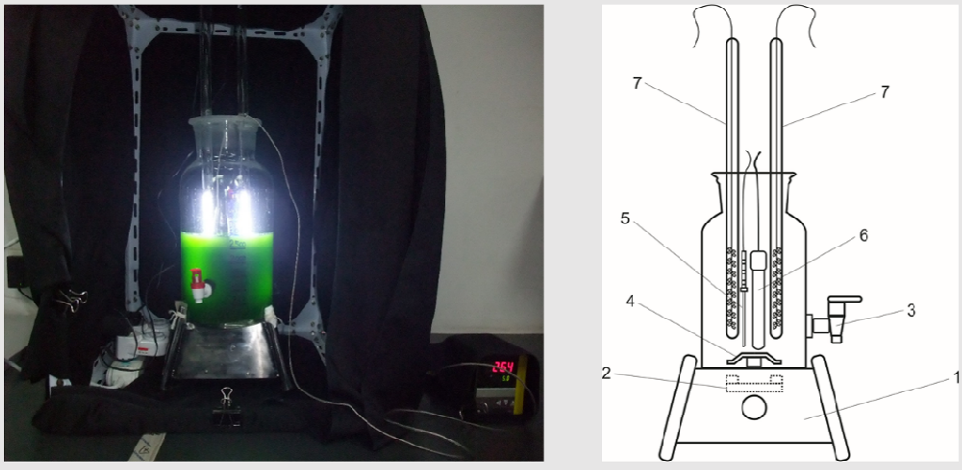

Fig.1. The set-up diagram of the algal reactor in the present study.

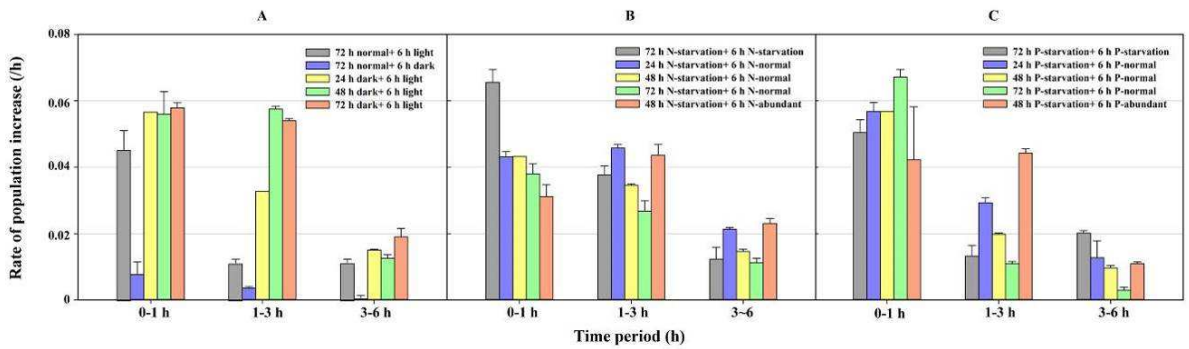

Fig.2. The rates of population increase (/h) of *C. pyrenoidosa* during the treatment when the alga underwent the artificial light-control culture (A), artificial N-control culture (B) and artificial P-control culture (C) before the treatment. Initial algal density: 11.00 × 10^6cell/mL; ceftazidime concentration: 40.00 mg/L. Values are the mean of three replicates in each treatment.

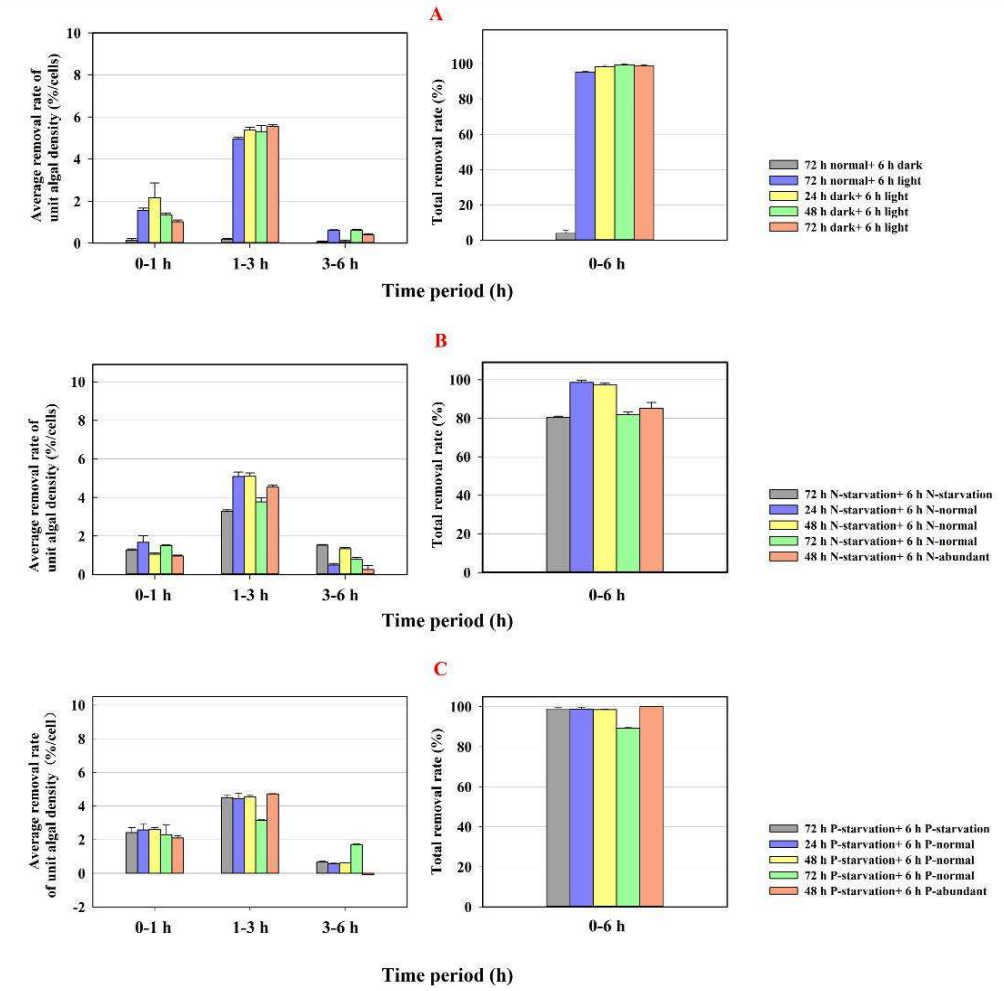

Fig.3. The average removal rates of ceftazidime by the unit cell and the total removal rate during the treatment when the algae underwent the artificial light-control culture (A), artificial N-control culture (B) and artificial P-control culture (C) before the treatment. Initial algal density: 11.00 × 10^6cell/mL; ceftazidime concentration: 40.00 mg/L. Values are the mean of three replicates in each treatment.

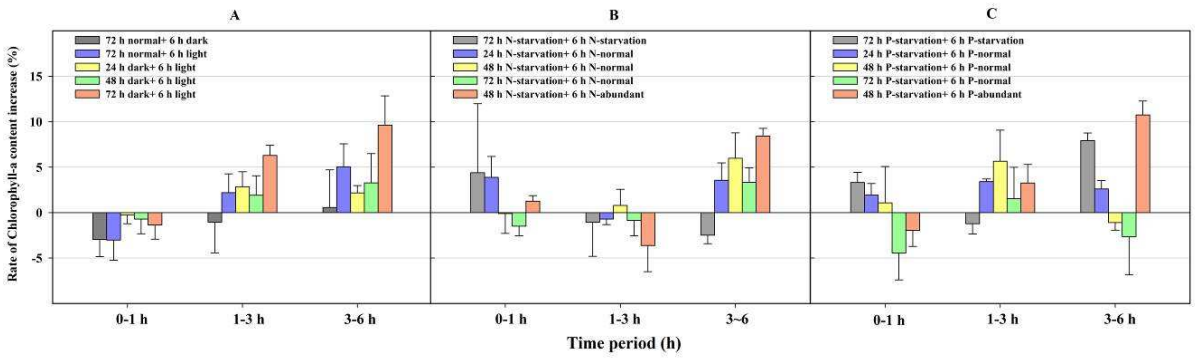

Fig.4. The rates of algal chlorophyll a content increase during the treatment when the alga underwent the artificial light-control culture (A), artificial N-control culture (B) and artificial P-control culture (C) before the treatment. Initial algal density: 11.00 × 10^6cell/mL; ceftazidime concentration: 40.00 mg/L. Values are the mean of three replicates in each treatment.

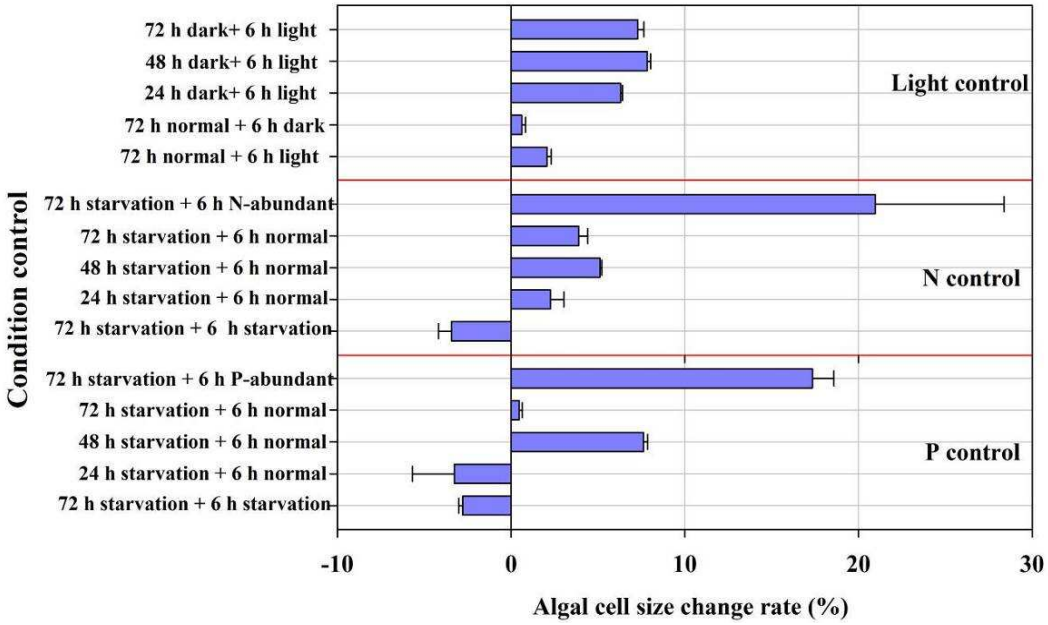

Fig.5. The change rates of the algal cell size during during the treatment when the alga underwent the artificial light-control culture (A), artificial N-control culture (B) and artificial P-control culture (C) before the treatment. Initial algal density: 11.00 × 10^6cell/mL; ceftazidime concentration: 40.00 mg/L. Values are the mean of three replicates in each treatment.

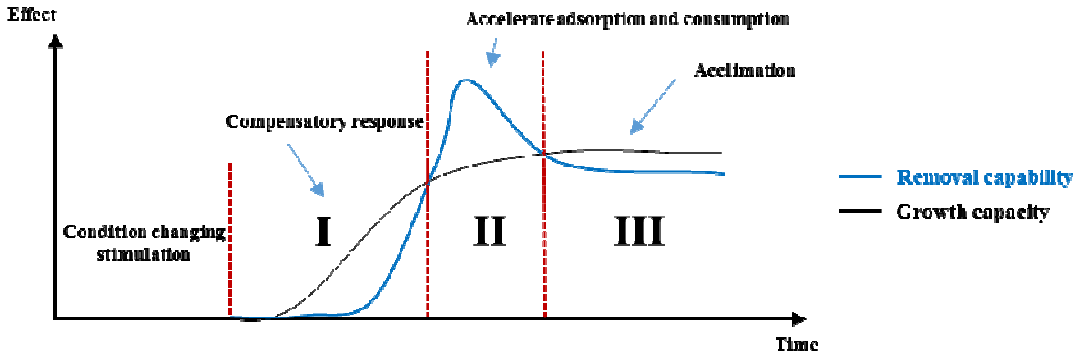

Fig.6. The possible three-step response model of the alga during the treatment.