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The Growth Behavior of *Chlorella vulgaris* in Bisphenol a Under Different Cultural Conditions

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Abstract

The effects of different initial concentrations of bisphenol A (BPA) on *Chlorella vulgaris* and removal capacity of BPA by *Chlorella vulgaris* were investigated under the light and the dark cultural conditions. Experiments were performed in 250 mL flasks under light and dark conditions with different BPA concentrations. Results showed that 0-20 mg·L^{-1} BPA concentration under the light condition and 0-10 mg·L^{-1} BPA concentration under dark condition plays a promoting role on the growth of *Chlorella vulgaris* in terms of cell density. The effect of BPA removal under light condition was obviously better than that under the dark condition. The maximum BPA removal rates were 3.425 ± 0.145 mg (L·d)$^{-1}$ and 1.530 ± 0.025 mg (L·d)$^{-1}$ under two conditions and were observed during 2-4 d and 0-2 d, respectively. The largest removal amounts of BPA under two conditions were all investigated in L-BPA$_{50}$ and D-BPA$_{50}$ groups. Both superoxide dismutase (SOD) and catalase (CAT) activities were promoted in all the treatments, which proved that *C. vulgaris* showed a positive response to the BPA stress condition. SOD activity showed sensitive and responsive to the new medium since it was promoted immediately on the incubation day. CAT activity was supposed to be more tightly controlled in response to BPA because its level was related to the BPA removal.

Keywords: *Chlorella vulgaris*; Bisphenol A; Light condition; Dark condition; Comparison

Introduction

With the global industrialization, production and usage of man-made substances in the industry have led to the entry of a wide variety of endocrine-disrupting chemicals into the environment [1]. Bisphenol A (BPA), which is made by Phenol and acetone [2], is an industrially important chemical that is used as a raw material in the manufacture of many products such as engineering plastics (e.g., epoxy resins/polycarbonate plastics), food cans (i.e., lacquer coatings), and dental composites/sealants [3]. Extensive evidence indicates that BPA induces feminization during gonadal ontogeny of fishes [4], reptiles [5], birds [6], and human [7,8], and it is identified as an endocrine disruptor and leads to carcinogenesis [9]. Biology is exposed to ubiquitous BPA Though its hazardous effects, more than 5 million metric tons of BPA was produced in 2011 and was mainly used in East Asia (Korea, China and Japan), and kept increasing year by year [10,11].

BPA is released into the environment mainly via two ways: sewage treatment effluent [12,13] and landfill leachate [14]. In the aquatic ecosystems, pollutants spread very quickly and have far-reaching consequences and particular attention should be paid to BPA. The previous study has even detected BPA in source water and drinking water [15]. The global level of BPA in most of the aquatic environments was lower than 1 μg/L [16]. BPA imposes deleterious effects on aquatic organisms, even at concentrations of less than 1 μg L^{-1} [17], making its detection and removal to non-toxic level a primary concern in water quality management.

Methods to remove BPA in the liquid phase include photo-degradation [16,18,19], oxidation [20-22], bacteria biodegradation [23-25], fungi biodegradation [26,27]. The knowledge on the biodegradation of BPA toward algal growth is of great importance due to its role in natural water bodies as a major primary producer, maintaining the balance of the aquatic ecosystem and are known to be comparatively sensitive to chemicals [28]. *Chlorella* is also one of the most tolerant eight genera [29] and highly tolerant to soluble organic compounds [30-33]. Wang et al. has identified novel pathways for biodegradation of BPA by green alga, while the maximum initial exposure concentration was low to inhibited algal growth [34]. Ji et al. used two stains of fresh microalgae for the biodegradation of BPA and utilization of algae under light condition [35]. Green alga *Monoraphidium braunii* was cultivated in the mixed medium of different level of BPA and natural organic matter, but the incubation time lasted relatively short (4 days) [36].

C. vulgaris can grow under photoautotrophic and heterotrophic conditions and algae tended to accumulate more biomass and grow faster in the organic carbon-rich medium under dark conditions [37-39]. Hence in our study, *C. vulgaris* was cultivated with different initial BPA concentrations under two cultivation conditions (mixotrophic and heterotrophic) to identify the interrelationship of BPA and algae. The biodegradation of BPA by algae and the influence of BPA on algal growth would be investigated. The characteristics of algae at different growth phases and algal stress enzymes were analyzed.

Materials and Methods

Chemical

Bisphenol A [2,2-(4,4-dihydroxydiphenyl) propane, 99% purity] was purchased from the Aladdin Chemistry Co., Ltd. (Shanghai,

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China). HPLC grade methanol (MeOH, 99.9%) was supplied by J&K Scientific Ltd. (Beijing, China) and ultrapure water was prepared in the laboratory using an ELGA ultrapure water machine (including water column), England. Other chemicals were used in analytical reagent grade and provided by the Shanghai LingFeng Chemical Reagent Co., Ltd. (Shanghai, China) and Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

C. vulgaris strain and pre-culture conditions

C. vulgaris (FACHB-31) used in this study was provided by the Chinese Academy of Sciences, Wuhan Institute of Aquatic Organisms. Then it was preserved in the BG-11 medium (Table 1) and cultivated in a 250 mL flask containing 100 mL growth medium to obtain a sufficient amount of cells; temperature was controlled at 25 ± 2°C, and light density was controlled at 2000 lux (the ratio of light to dark was 14:10) in Boxun light growth chamber (SPX-250B-G, Shanghai Boxun Industry & Commerce Co., Ltd., China). This sample was used throughout the study.

Experimental procedure

Cultivation medium: The basic medium was BG11 medium, which also was set as the control group. Five initial BPA concentrations (2, 5, 10, 20, 50 mg·L^{-1}) were added in the basic medium for the algal cultivation. Experiments were performed using a 500 mL flask with 250 mL of working volume. Reactors were incubated with *C. vulgaris* obtained from a stock *C. vulgaris* reactor to produce an initial cell density of approximately 5×10^6 mL^{-1}.

Light condition: The reactors were illuminated in a light growth chamber with 2000 lux light intensity (the ratio of light to dark was 12:12) and 25 ± 2°C temperature for 10 d. The BPA groups under this condition were set as L-BPA$_0$, L-BPA$_2$, L-BPA$_5$, L-BPA$_{10}$, L-BPA$_{20}$, and L-BPA$_{50}$.

Dark condition: Five glucose concentrations (1 g·L^{-1}, 2 g·L^{-1}, 5 g·L^{-1}, 10 g·L^{-1}) were tested for the heterotrophic cultivation of *C. vulgaris* in the previous experiment (statistics were not shown). Considering both benefiting algal growth and limiting the residual glucose at the end of cultivation, 1 g·L^{-1} was chosen for *C. vulgaris* cultivation. The reactors were put in a thermostatic incubator at 25 ± 2°C temperature for 10 d. The BPA groups under this condition were set as D-BPA$_0$, D-BPA$_2$, D-BPA$_5$, D-BPA$_{10}$, D-BPA$_{20}$, and D-BPA$_{50}$.

Sample preparation and analysis

Before sampling was conducted, the biomass attached to the reactor walls was carefully suspended by swirling the culture contents. At different time intervals (mainly on 0, 2, 4, 6, 8, 10 d), approximately 40 mL of the samples were removed from the reactors to monitor the biomass growth in terms of cell density and stress enzymes. The left aqueous phase was gathered to determine residual BPA concentration. All the experiments were performed in triplicate, and average values were recorded.

Chemicals	Content g/L	Chemicals	Content g/L
$NaNO_3$	1.5 g	$K_2HPO_4·3H_2O$	40 mg
$MgSO_4·7H_2O$	75 mg	$CaCl_2·2H_2O$	36 mg
Citric acid	6 mg	Ferric ammonium citrate	6 mg
$Na_2EDTA·2H_2O$	1 mg	Na_2CO_3	20 mg
H_3BO_3	2.86 mg	$MnCl_2·4H_2O$	1.81 mg
$ZnSO_4·7H_2O$	0.22 mg	$Na_2MoO_4·2H_2O$	0.39 mg
$CuSO_4·5H_2O$	0.079 mg	$Co(NO_3)_3·6H_2O$	0.0494 mg

Table 1: The components of BG11.

Measurement of *C. vulgaris* cell growth: *C. vulgaris* proliferation was determined by direct counting by using a Neubauer hemocytometer under an optical microscope (BA200; Shanghai Boxun Industry & Commerce Co., Ltd., China) with eyepiece (10 times) and objective (40 times).

Chlorophyll-a analysis: Chlorophyll-a was measured after extraction with methanol. Samples of *C. vulgaris* were centrifuged (5000 rpm, 10 min), washed twice by deionized (DI) water, and the pellet was resuspended in 8 mL of 100% methanol and disrupted in an ultrasonic cleaner (A NA1860, Yingsum Ultrasonic Equipment Co., Ltd., China) at 135 W with ice bag in the dark place for a duration of 40 minutes. After chlorophyll-a extraction, samples were centrifuged (5000 rpm, 10 min); the amount of chlorophyll-a in the supernatants was diluted with methanol to 10 mL and absorbance was measured at 653 and 666 nm. Chlorophyll-a concentration was calculated according to Ritchie's and Zheng's method [40,41].

$$C_a=[15.65 \times OD666-7.34 \times OD653] \times \text{Dilution Ratio} \quad (1)$$

Where, C_a stands for Chlorophyll-a concentration and OD stands for absorbance which measured at 653 and 666 nm.

BPA analysis: BPA contents in the aqueous phase were measured by high-performance liquid chromatography (HPLC, LC- 10ATVP, Kyoto, Japan) using a reversed-phase C-18 column (250 nm × 4.6 nm, 5 μm) as the stationary phase and a mixture of methanol and H_2O (77:23) as the mobile phase. The flow rate was maintained at 1 mL min^{-1} and a wavelength of 280 nm was used.

preparation of enzymes extracts and activity analysis: For preparing extracts, 25 mL *C. vulgaris* sample was centrifuged (5000 rpm, 10 min), washed twice with phosphate-buffered saline (PBS; pH=7), and the pellet was resuspended in 5 mL PBS. Next, the suspension was disrupted using a 300 W ultrasonic processor (Fs-300; Shanghai Sonxi Co., Ltd., China) for 4 s at 4 s intervals for a duration of 20 min in an ice-water bath to allow the intracellular substances to move out of the cells and enter the liquid phase. The sample was then centrifuged at 20,000 rpm for 5 min to obtain the supernatant as enzyme extract. All the steps in enzyme extract preparation were performed at 4°C. The extract was used to measure the activities of antioxidant enzymes.

Superoxide dismutase (SOD) activity was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) and the change in absorbance were measured at 560 nm [42]. The reaction mixture consisted of 25 mM phosphate buffer (pH 7.8), 65 μM NBT, 2 μM riboflavin, enzyme extract, and TEMED and the reaction mixture was exposed to the light of 350 μmol m^{-2} s^{-1} for 15 min. To determine the SOD activity in per 10^6 algal cells indicated in this paper, we divided the SOD activity by the corresponding cell density.

Catalase analysis: Catalase (CAT) was measured with $KMnO_4$ titration method. 5 mL algal suspension was mixed thoroughly with 15 mL distilled water and 2.5 mL hydrogen peroxide (0.1 M). The sample was then incubated for 30 min at 120 r min^{-1} and 35 ; the reaction was stopped by adding 2.5 mL of sulfuric acid (1.5 M). After the mixture was filtered, 25 mL filtrate was acquired and titrated using 10 μM $KMnO_4$ until it turned to pink (do not fate after 20 s). CAT activity (U) was calculated according to the following Eq. (33):

$$M(U)=(V0-V)\ /W \times C \quad (2)$$

Where, M is the CAT activity, Vo is the consumption volume of $KMnO_4$ for the blank sample (without algae), V is the consumption volume of $KMnO_4$ for the control sample (with algae), W is the quality of activated sludge, and C is the concentration of $KMnO_4$.

Statistical analysis

All data were expressed as means ± standard error of the mean (SEM). Statistical analysis was performed using IBM SPSS 22.0 (IBM Corporation, Somers, NY). Analysis of variance (ANOVA/MANOVA) was used to determine the significance of differences between the groups. The Pearson correlation test was performed for determining the correlations between the parameters. The level of statistical significance was set at $p<0.05$.

Results and Discussion

The growth of *C. vulgaris* under two conditions

The variations of cell density of *C. vulgaris* in different initial BPA concentration under two cultivation conditions and their corresponding growth rates were shown in Figure 1. The first 2 days was investigated to be the lag phase of algal growth since cell density of every group remained at the beginning level and the growth rates were at a very low level under light condition. From the 4th day, differences between each group appeared and became obvious with time. Considering the growth tends, period 4-8 d was supposed to be the log phase of *C. vulgaris* growth. The cell density of L-BPA_0 group (control group) reached (23.17 ± 0.98) × 10^6 mL^{-1} and the highest cell density was obtained by L-BPA5 group ((38.25 ± 1.38) × 10^6 mL^{-1}), while L-BPA_{50} group only gained (7.44 ± 0.17) × 10^6 mL^{-1} on the 8th day. BPA could stimulate the growth of *C. vulgaris* in terms of cell density when the concentration was under 20 mg L^{-1} and the cell densities of L-BPA_2, L-BPA_5, and L-BPA_{10} groups ((33.25 ± 1.50) × 10^6 mL^{-1}, (38.25 ± 1.38) × 10^6 mL^{-1}, (35.92 ± 1.58) × 10^6 mL^{-1}) were significantly higher than the control group ($p<0.01$). This might because the low concentration of BPA was taken as the organic substance resources by algae for growth. The growth of green alga *Monoraphidium braunii* also was promoted by under 4 mg/L BPA [36]. The growth rates of L-$BPA_{2,5,10}$ groups were at the similar level with the control group during 2-6 d, while obviously higher than the control group ($p<0.01$) on the 8th day. The growth rate of L-BPA_{20} group was lower than the control group from 2-6 d, but higher during 6-8 d period. 20 mg L^{-1} BPA might be a "critical" concentration for *C. vulgaris* growth. Algae was firstly inhibited and the log phase was prolonged, but finally, could recover and acclimate itself to the toxic circumstance. In Ji's study, 25 mg L^{-1} inhibited BPA concentration was also found on the growth of both *Chlamydomonas mexicana* and *Chlorella vulgaris* [35]. A similar phenomenon was shown when cultivating *C. vulgaris* in 4-chlorophenol and 2,4-dichlorophenol, which also had an inhibition concentration for algal growth [31]. When using microalgae consortium to biodegrade p-chlorophenol (PCP), the lag phase of algae was prolonged from 12 ± 1.8 d to 14.7 ± 1.2 d as the initial PCP concentration was increased from 100 mg L^{-1} to 150 mg L^{-1} [43]. The rest time (8-10 d) was considered as the stable phase of the algal growth and the growth rate showed the algae death. The growth of *C. vulgaris* was totally inhibited under 50 mg L^{-1} BPA concentration and the growth rate was lower than the control group along the cultivation ($p<0.01$).

Different from the light condition, there was no obvious lag phase in *C. vulgaris* growth except D-BPA_{50} group under the dark condition with 1 mg L^{-1} glucose. The first 4 days was considered as the log phase since the algae grew rapidly and reached the optimum. After the stable phase (4-6 d) with the growth rate around zero, algae came into the decline phase (6-10 d) with the growth rate less than zero. Compared with the light condition, the addition of BPA (under 10 mg L^{-1}) also would not inhibit the growth of algae, but the beneficial effect was not obvious as that under the light condition, for the cell density relatively higher than the control group. What's more, the highest cell density gained by D-BPA_5 ((27.08 ± 1.22) × 10^6 mL^{-1}) was about 11 × 10^6 mL^{-1} fewer than that of L-BPA5 ((38.25 ± 1.38) × 10^6 mL^{-1}). The "critical" concentration was 10 mg L^{-1} under the dark condition which also lower that of light condition. The peak cell density of D-BPA_{50} group ((10.98 ± 0.44) × 10^6 mL^{-1}) appeared on 4th day, which was less than half of the control group's ((24.46 ± 1.18) × 10^6 mL^{-1}). Though the growth rate of *C. vulgaris* in dark condition showed faster growth trending during the first 4 days, the highest growth rate (D-BPA_5: (41.84 ± 3.41) × 10^5 $(L{\cdot}d)^{-1}$, 0-2 d) which much lower than that of the light condition (L-BPA5: (124.92 ± 11.02) × 10^5 $(L{\cdot}d)^{-1}$, 6-8 h). Light is a very important parameter for

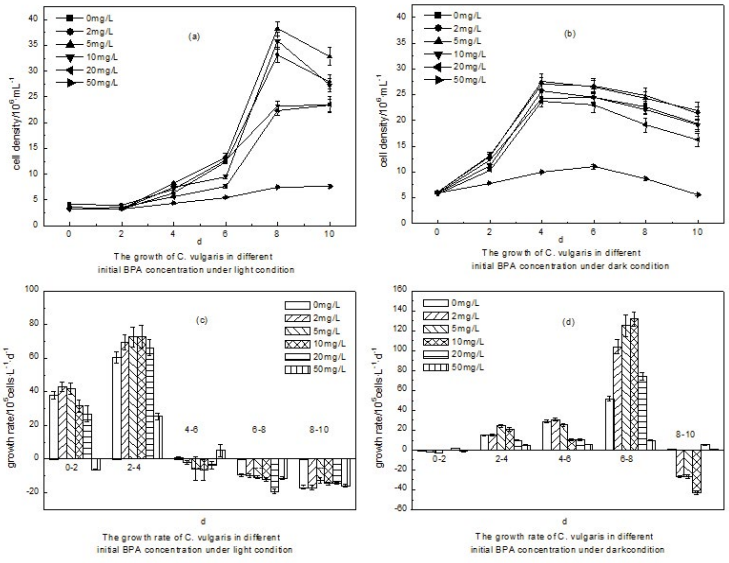

Figure 1: (a) The growth of *C. vulgaris* in different initial BPA concentration under light condition (b) the growth of *C. vulgaris* in different initial BPA concentration under dark condition (c) the growth rate of *C. vulgaris* in different initial BPA concentration under light condition (d) the growth of *C. vulgaris* in different initial BPA concentration under light condition.

microalgae growth. Microalgae consortium could biodegrade more p-chlorophenol under light condition [43]. Under light conditions, *C. vulgaris* tended to have higher BPA tolerable concentration and gain more biomass during the cultivation than the dark condition.

The removal of BPA under two conditions

The removal and residual of BPA under light and dark conditions after 10-days cultivation was shown in Figure 2. BPA removal amount by *C. vulgaris* under light condition increased along with the increase of initial BPA concentration and the maximum removal amount appeared in the L-BPA_{50} group (15.79 mg L^{-1}). BPA removal under dark condition showed the similar tendency and the maximum removal amount also appeared in D-BPA_{50} group (7.30 mg L^{-1}), which only half of the light condition. Table 2 shows the BPA removal value and ratio under two cultivation conditions. The final BPA removal amount has good correlation with initial BPA concentrations under cultivation conditions, 0.992 ($p<0.01$) under light condition and 0.989 ($p<0.01$) under dark condition, respectively. The light condition would benefit the removal of BPA, especially when the initial BPA concentration up 10 mg L^{-1}.

The BPA removal rates during every sampling period under two cultivation conditions has been shown in Figure 3. The removal rate differences between each time period of L-BPA_2 and L-BPA_5 groups were not evident, while the differences became obvious when the initial BPA concentration up to 10 mg L^{-1}. The removal rates of BPA_{20} and BPA_{50} during 2-4 d were 1.325 ± 0.074 and 3.425 ± 0.145 mg $(L{\cdot}d)^{-1}$ respectively, which was significantly higher than other time period ($p<0.01$). According to our previous discussion, period 2-4 d was the beginning phase of algal log growth period but not the optimal growth rate period (6-8 d). The BPA removal rates of every group under dark condition were relatively faster during period 0-2 d, which also was the beginning phase of algal log period. The highest removal rate under dark condition was 1.530 ± 0.025 mg $(L{\cdot}d)^{-1}$ of L-BPA_{50}, which also much lower than that under light condition. Whatever, the highest BPA removal rates under two cultivation conditions were both shown in BPA_{50} group during the initial stage of the log phase of cultivation. A good correlation could be observed between both the removal rate during 2-4 d under the light condition and 6-8 d under the dark condition with the initial BPA concentration (0.998, $p<0.001$; 0.982, $p<0.001$). In the study of the biodegradation of p-chlorophenol by a microalgal consortium, the researchers also found that the duration of the lag phase corresponded to the time needed for complete p-CP degradation to occur [43]. What's more, though the percentage of residual BPA would increase as the initial BPA concentration increased, the absolute removal amount increased [36].

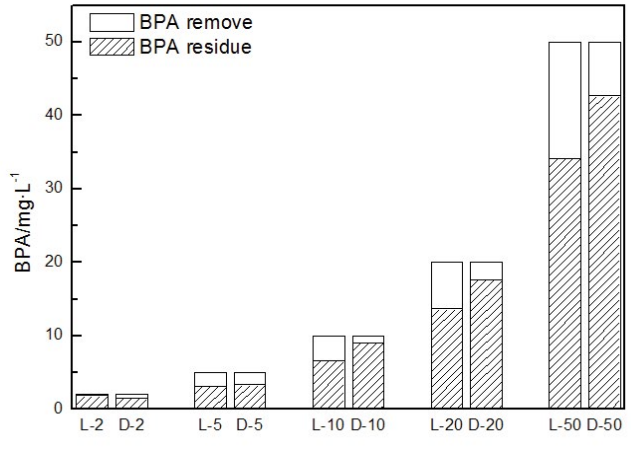

Figure 2: Comparison of the removal of BPA under two cultivation conditions.

SOD and CAT analysis

Toxic chemicals and stress conditions would result in oxidative damage to algae [44,45] by overproduction of reactive oxygen species (ROS), including superoxide radical (O_2^-), singlet oxygen (O_2^1), hydrogen peroxide (H_2O_2), and hydroxyl radical (HO^-) [46]. The SOD-CAT system is the first line of defense of the body against oxidative stress and can be used as a biomarker of ROS production [47]. In our study, the SOD activity and CAT activity in per 10^6 algal cells were investigated in order to analyze the *C. vulgaris* response to the different concentration of BPA and deeper analysis of BPA removal.

Effect of BPA on SOD activity of *C. vulgaris*: SOD acts as antioxidants to against the superoxide radicals and protects cellular components from being oxidized by ROS [48,49]. The effects of BPA on SOD activity of *C. vulgaris* under two conditions were shown in Figure 4. Compared with CAT activity under the light condition, SOD activity was more sensitive and responsive to the new cultivation. The SOD activity of every group was stimulated when incubated to the new medium, even the control group's algae secreted more enzyme (over 4 U/10^6 cells) on the incubation day. Then the control group's SOD activity remained at 2 U/10^6 cells level the rest time of the cultivation. Compared with control group, the SOD activity of BPA groups was obviously promoted by the increasing initial BPA concentration on the incubation day ($p<0.05$) and on the 2nd day ($p<0.01$). Period 0-2 d was the lag phase of algal growth and SOD activity reacted immediately to the stress condition. The SOD activity level of L-BPA_2 and L-BPA_5 groups dropped to control groups level on the 4th day, L-BPA_{10} on the 6th day and L-BPA_{20} on the 8th day. While the SOD activity level of L-BPA_{50} was higher than the control group along the cultivation time.

Under dark condition, the SOD activity in *C. vulgaris* of every group also showed more sensitive and responsive reaction to the new medium than CAT activity since the SOD activity of every group was promoted on the incubation day. This promotion also lasted during the 0-2 d. Different from the light condition, the SOD activity level of D-BPA_{20} and D-BPA_{50} didn't drop to the control group's level until the 6th day. This phenomenon also supports that the "critical" concentration for *C. vulgaris* under dark condition would be 10 mg L^{-1}. What's more, the maximum SOD activity level was 5.05 U/10^6 cells (D-BPA_{50}, 0 d), which was lower than that under light condition (8.44 U/10^6 cells, L-BPA_{50}, 0 d). We supposed that the light condition stimulated the SOD activity which contributed to the algal ability to overcome the stress condition.

Influence of different concentration of BPA on enzymes activity: CAT is an enzyme present in the peroxisomes and mitochondria where it decomposes H_2O_2 into water and oxygen [49]. Increase in CAT activity is believed to maintain the H_2O_2 steady-state level within the cells. The acute and chronic effects of BPA on CAT activity of per 10^6 algal cells are shown in Figure 5. Under the light condition, the CAT activity of the control group was about 0.2 U during the whole cultivation period. The CAT activities of BPA groups were at the similar level (around 0.2 U) with the control group at the incubation day. While the CAT activities of BPA groups were observed significantly higher than the control group on the 2nd day ($p<0.01$). The CAT activity in per 10^6 algal cells stimulated along with the increase of the initial BPA concentration. The CAT activity of BPA groups decreased at 96 h, but

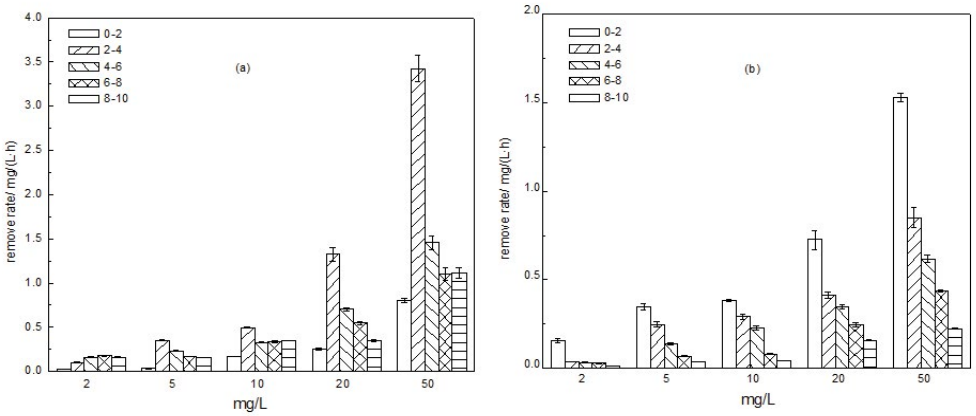

Figure 3: BPA removal rate per unit time (a. Light; b Dark).

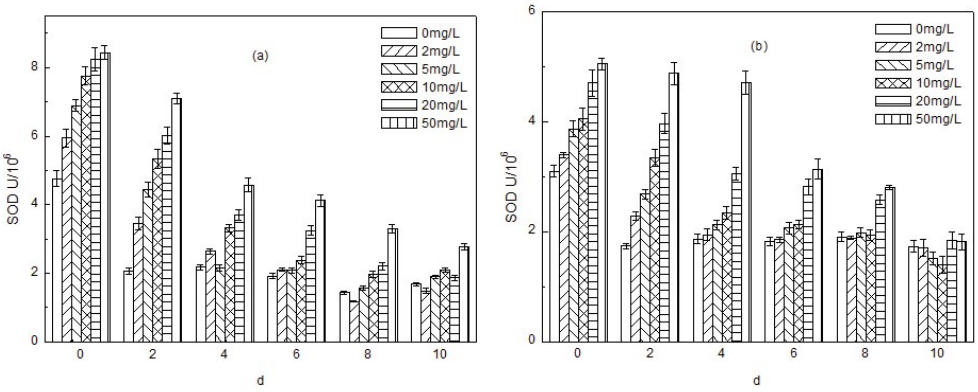

Figure 4: SOD activities of *C. vulgaris* during different periods under various concentrations of BPA.

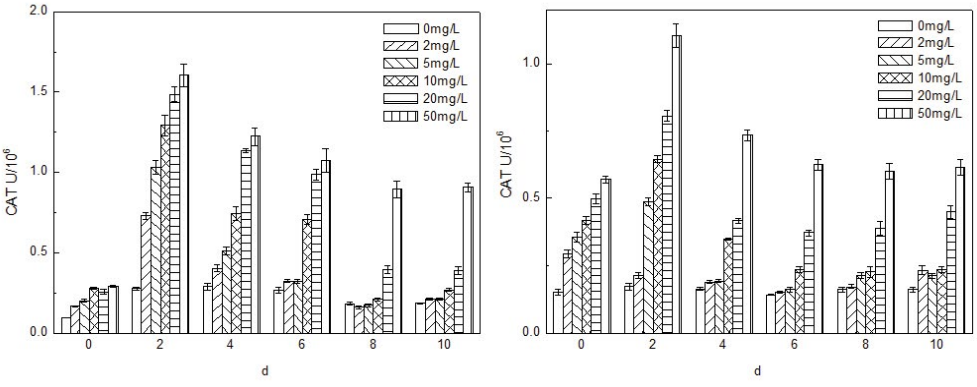

Figure 5: CAT activities of *C. vulgaris* during different periods under various concentrations of BPA.

they still remain at higher levels compared to the control group. As we discussed above, the beginning phase of algal log growth period (2-4 d) gained the highest BPA removal rate, which might be supposed the removal of BPA by algae promoted the CAT activity in the algal cells. Along with the cultivation, the CAT activity of L-BPA$_{10}$, L-BPA$_{20}$ and L-BPA$_{50}$ were still higher than the control group ($p<0.01$) while that of L-BPA$_{2}$ and L-BPA$_{5}$ group were decreased to the level of the control group. The CAT activity of L-BPA10 and L-BPA$_{20}$ reduced to the control group's level on the 6th day and 10th day, respectively, while that of L-BPA 50 remained obviously higher than the control group's. CAT activities of BPA groups decreased in the later stages might due to two reasons: 1) metabolism and oxidative stress of *C. vulgaris* could manage toxicity in a culture, time-dependent manner [50]. 2) BPA was biodegraded by algae and the stimulation of residual BPA to algae was correspondingly lower.

	Light condition				
Removal value	1.24	1.86	3.35	6.33	15.79
Removal ratio	0.62	0.372	0.335	0.3165	0.3158
	Dark condition				
Removal value	0.51	1.65	2.03	3.76	7.3
Removal ratio	0.255	0.33	0.203	0.188	0.146

Table 2: BPA removal value and ratio under two cultivation conditions.

Under dark condition, Compared with the light condition, the CAT activities of BPA groups could be observed higher than the control group's at the incubation day and this trend lasted until 48 h, while this period (0-48 h) was also the beginning part of algal log growth phase under dark condition. Different from the light condition, the CAT activity of D-BPA_{20} didn't decrease to the control group's level, which also proved that 20 mg L^{-1} was the "critical" concentration under the light condition and 10 mg L-1 under dark condition. What's more, the maximum CAT activity of L-BPA_{50} group and D-BPA_{50} groups was 1.61 U and 1.10 U, respectively. So we supposed that light condition was conducive to the stimulation of CAT in algae cells to overcome the stress condition.

Increased SOC and CAT activity could be considered indirect evidence of enhancing production according to Mittler' study [49]. In Chen's study, SOD and CAT activities of *C. vulgaris* increased during 24-120 h exposure to sodium pentaborate pentahydrate, but gradually decreased as culture time progressed [50]. SOD and CAT activities of both *Chlorella pyrenoidosa* and *Scenedesmus obliquus* were promoted in the treatments of different concentration of BPA according to Zhang's research [51]. In our study, we supposed that addition of BPA caused oxidative damage to *C. vulgaris* and SOD activity in algal cells was considered as the response to this damage. Though the SOD and CAT activity calculated in the total amount at the different time point were investigated that would be promoted by the toxic chemicals, the analysis would be more accurate if the enzymes were analyzed in per unit cells. What's more, SOD activity showed a more sensitive response to BPA stress condition than CAT activity. The enzyme in per 10^6 algal cells helps us to identify that CAT activity acted tightly with optimal BPA removal rate period.

Conclusions

The *C. vulgaris* was cultivated in different initial concentration BPA under light and dark conditions for both BPA removal and algal growth analysis. Algal growth could be promoted by the low concentration of BPA (light: 20 mg L^{-1}; dark: 10 mg L^{-1}) in term of cell density. Algae tended to remove more BPA under light condition than the dark condition. The optimal BPA removal rates were observed during the early stage of algal log growth phase under both conditions. The removal of BPA by *C. vulgaris* increased as the initial BPA concentration increase. Enzymes in per 10^6 algal cells investigated that both SOD and CAT activities were promoted in all the treatments, which proved that algae could respond to the increasing concentration of BPA by secreting more stress enzymes. The enzymes level were tightly controlled in response to BPA and related to the BPA removal.

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