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-BPA₀ and D-BPA₀ 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 photodegradation [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, China).

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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 × 10⁶ 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-BPA₀, L-BPA₂, L-BPA₅, L-BPA₁₀, L-BPA₂₀, and L-BPA₅₀.

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-BPA₀, D-BPA₂, D-BPA₅, D-BPA₁₀, D-BPA₂₀, and D-BPA₅₀.

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.

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, Yinghua 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].

\[
C_v = \frac{15.65 \times OD_{665} - 7.34 \times OD_{653}}{\text{Dilution Ratio}}
\]

Where, \(C_v\) 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 H₂O (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 Ultrasonic Equipment 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 10⁶ algal cells indicated in this paper, we divided the SOD activity by the corresponding cell density.

Catalase analysis: Catalase (CAT) was measured with KMnO₄ 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°C, 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 KMnO₄ until it turned to pink (do not fate after 20 s). CAT activity (U) was calculated according to the following Eq. (33):

\[
M(U) = \frac{(V₀ - V) \times W}{C}
\]
Where, M is the CAT activity, Yo is the consumption volume of K\textsubscript{MnO}\textsubscript{4} for the blank sample (without algae), V is the consumption volume of K\textsubscript{MnO}\textsubscript{4} for the control sample (with algae), W is the quality of activated sludge, and C is the concentration of K\textsubscript{MnO}\textsubscript{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\textsubscript{5} group (control group) reached $(23.17 ± 0.98) \times 10^6$ mL\textsuperscript{-1} and the highest cell density was obtained by L-BPA\textsubscript{5} group ($(38.25 ± 1.38) \times 10^6$ mL\textsuperscript{-1}), while L-BPA\textsubscript{50} group only gained $(7.44 ± 0.17) \times 10^6$ mL\textsuperscript{-1} on the 8th day. BPA could also be promoted by *C. vulgaris* 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 was set at p<0.05.

The growth of *C. vulgaris* concentration for 4 mg/L BPA [36]. The growth rates of L-BPA2,5,10 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\textsubscript{5} group was lower than the control group from 2-6 d, but higher during 6-8 d period. 20 mg L\textsuperscript{-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\textsuperscript{-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\textsuperscript{-1} to 150 mg L\textsuperscript{-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\textsuperscript{-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\textsubscript{5} group under the dark condition with 1 mg L\textsuperscript{-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\textsuperscript{-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\textsubscript{5} ($(27.08 ± 1.22) \times 10^6$ mL\textsuperscript{-1}) was about $11 \times 10^6$ mL\textsuperscript{-1} fewer than that of L-BPA\textsubscript{5} ($(38.25 ± 1.38) \times 10^6$ mL\textsuperscript{-1}). The “critical” concentration was 10 mg L\textsuperscript{-1} under the dark condition which also lower that of light condition. The peak cell density of D-BPA\textsubscript{5} group ($(10.98 ± 0.44) \times 10^6$ mL\textsuperscript{-1}) appeared on 4th day, which was less than half of the control group's ($(24.46 ± 1.18) \times 10^6$ mL\textsuperscript{-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\textsubscript{5} $(41.84 ± 3.41) \times 10^5$ (L·d)$^{-1}$, 0-2 d) which much lower than that of the light condition (L-BPA5: $(124.92 ± 11.02) \times 10^5$ (L·d)$^{-1}$, 6-8 h). Light is a very important parameter for

![Figure 1](image-url)
microalgal growth. Microalgal 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 dark condition increased along with the increase of initial BPA concentration and the maximum removal amount appeared in the L-BPA10 group (15.79 mg L\(^{-1}\)). BPA removal under dark condition showed the similar tendency and the maximum removal amount also appeared in D-BPA10 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\(_0\), and L-BPA\(_{10}\) 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·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·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].

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\(_2\)), hydrogen peroxide (H\(_2\)O\(_2\)), and hydroxyl radical (HO\(_\cdot\)) [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 2\(^{nd}\) 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\(_{10}\) and L-BPA\(_{50}\) groups dropped to control groups level on the 4\(^{th}\) day, L-BPA\(_{10}\) on the 6\(^{th}\) day and L-BPA\(_{50}\) on the 8\(^{th}\) 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 6\(^{th}\) 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\(_2\)O\(_2\) into water and oxygen [49]. Increase in CAT activity is believed to maintain the H\(_2\)O\(_2\) 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 2\(^{nd}\) 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
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-BPA10, L-BPA20 and L-BPA50 were still higher than the control group (p<0.01) while that of L-BPA2 and L-BPA5 group were decreased to the level of the control group. The CAT activity of L-BPA10 and L-BPA50 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. CAT activities of BPA groups decreased in the later stages might due to two reasons: 1) metabolism and oxidative stress of Chlorella 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.

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

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

Figure 5: CAT activities of Chlorella vulgaris during different periods under various concentrations of BPA.
Under dark condition, compared with the light condition, the CAT activity of BPA groups could be observed higher than 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\(_{20}\) group and D-BPA\(_{20}\) 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 enhanced production according to Mittler's 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 algae 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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References

1. Mendes JA (2002) The endocrine disruptors: a major medical challenge. Food and Chemical Toxicology 40: 781-788.
2. Brunnelle DJ (2005) Advances in Polycarbonates: An Overview. ACS Symposium Series, pp: 1-5.
3. Huang YQ, Wong CK, Zheng JS, Bouwman H, Barra R, et al. (2012) Bisphenol A (BPA) in China: a review of sources, environmental levels, and potential human health impacts. Environmental International 42: 91-99.
4. Mita L, Blanco M, Viggiano E, Zollo F, Bencivenga U, et al. (2011) Bisphenol A content in fish caught in two different sites of the Tyrrhenian Sea (Italy). Chemosphere 82: 405-410.
5. Marshack LK, Conard CM, Bryan SJ, Deem SL, Holliday DK, et al. (2017) Transcriptomic alterations in the brain of painted turtles (Chrysemys picta) developmentally exposed to bisphenol A or ethynyl estradiol. Physiological Genomics 49: 201-215.
6. Hallidin K, Berg C, Bergman Å, Brandt I, Brunström B (2001) Distribution of bisphenol A and tetramerosobiphenol A in egg yolk, embryos and laying birds and studies on reproduction variables in adults following in ovo exposure. Archives of Toxicology 75: 597-603.
7. Takayanaagi S, Tokunaga T, Liu X, Okada H, Matsuhashi A, et al. (2006) Endocrine disruptor bisphenol A strongly binds to human estrogen-related receptor y (ERRy) with high constitutive activity. Toxicology Letters 167: 95-105.
8. Takeshita A, Koibuchi N, Oka J, Taguchi M, Shishita Y, et al. (2001) Bisphenol-A, an environmental estrogen, activates the human orphan nuclear receptor, steroid and xenobiotic receptor-mediated transcription. European Journal of Endocrinology 145: 513-517.
9. Crain DA, Eriksen M, Uguchi T, Jobling S, Laufer H, et al. (2007) An ecological assessment of bisphenol-A: evidence from comparative biology. Reproductive Toxicology 22: 225-239.
10. Hoepner LA, Whitty RM, Just AC, Calafat AM, Perera FP, et al. (2013) Urinary concentrations of bisphenol A in an urban minority birth cohort in New York City, prenatal through age 7 years. Environmental Research 122: 38-44.
11. Yamazaki E, Yamashita N, Taniyasu S, Lam J, Lam PK, et al. (2015) Bisphenol A and other bisphenol analogues including BPS and BPF in surface water samples from Japan, China, Korea and India. Ecotoxicology and Environmental Safety 122: 565-572.
12. Hou G, Zhang R, Hao X, Liu C (2017) An explanation of the effect and interaction mechanism of bisphenol A on waste sludge hydrolysis with multi-spectra, isothermal titration microcalorimetry and molecule docking. Journal of Hazardous Materials 333: 32-41.
13. Meesters RJ, Schröder HF (2002) Simultaneous determination of 4-nonylphenol and bisphenol A in sewage sludge. Analytical Chemistry 74: 3566-3574.
14. Wintgens T, Galletkemper M, Metin T (2003) Occurrence and removal of endocrine disruptors in landfill leachate treatment plants. Water Science and Technology 48: 127-134.
15. Kleywegt S, Pileggi Y, Yang P, Hao C, Zhao X, et al. (2011) Pharmaceuticals, hormones and bisphenol A in untreated source and finished drinking water in Ontario, Canada—occurrence and treatment efficiency. Science of the Total Environment 409: 1481-1488.
16. Huang W, Luo M, Wei C, Wang Y, Hanna K, et al. (2017) Enhanced heterogeneous photo-Fenton process modified by magnetite and EDDS: BPA degradation. Environmental Science and Pollution Research 24: 10421-10429.
17. Oehlmann J, Schulte-Oehlmann U, Bachmann J, Otken M, Lutz I, et al. (2006) Bisphenol A induces supermethylazeration in the ramshorn snail (Gastropoda: Prosobranchia) at environmentally relevant concentrations. Environmental Health Perspectives 114: 127.
18. Bechambi O, Jaisel L, Najjar W, Sayadi S (2016) Photocatalytic degradation of bisphenol A in the presence of Ce-ZrO2: Evolution of kinetics, toxicity, and photocatalysis degradation mechanism. Materials Chemistry and Physics 173: 95-105.
19. Zhou Y, Gu X, Zhang R, Lu J (2015) Influences of various cyclodextrins on the photodegradation of phenol and bisphenol A under UV light. Industrial & Engineering Chemistry Research 54: 426-433.
20. Chen Z, Zhang Y, Zhou L, Zhu H, Wan F, et al. (2017) Performance of nitrogen-doped graphene aerogel particle electrodes for electro-catalytic oxidation of simulated Bisphenol A wastewaters. Journal of Hazardous Materials 332: 70-78.

21. Han Q, Wang H, Dong W, Liu T, Yin Y, et al. (2015) Degradation of bisphenol A by ferrate (VI) oxidation: Kinetics, products and toxicity assessment. Chemical Engineering Journal 262: 34-40.

22. Potakis N, Frontistis Z, Antonopoulou M, Konstantinou I, Mantzavinos D (2017) Oxidation of bisphenol A in water by heat-activated persulfate. Journal of Environmental Management 195: 125-132.

23. Chen X, Zhao J, Zhao J, Yang N, Zhang F, et al. (2014) The influence of SBR parameters on the sludge age of synthetic wastewater containing bisphenol A. Environmental Science and Pollution Research 21: 9287-9296.

24. Xie YT, Li HB, Wang L, Liu Q, Shi Y, et al. (2011) Molecularly imprinted polymer microspheres enhanced biodegradation of bisphenol A by acclimated activated sludge. Water Research 45: 1189-1198.

25. Zhao J, Chen X, Lin F, Yang N, Huang H, et al. (2014) Mechanism of toxicity formation and spatial distribution in activated sludge treating synthetic effluent containing bisphenol A (BPA). Chemical Engineering Journal 250: 91-98.

26. Chai W, Handa Y, Suzuki M, Saito M, Kato N, et al. (2005) Biodegradation of bisphenol A by fungi. Applied Biochemistry and Biotechnology 120: 175.

27. Zhang C, Li M, Chen X, Li M (2015) Edible fungus degrade bisphenol A with no harmful effect on its fatty acid composition. Ecotoxicology and Environmental Safety 118: 126-132.

28. Abdel-Hamid MI (1996) Development and application of a simple procedure for toxicity testing using immobilized algae. Water Science and Technology 33: 129-38.

29. Abdel-Raouf N, Al-Homaidan AA, Ibraheem IB (2012) Microalgae and wastewater treatment. Saudi Journal of Biological Sciences 19: 257-275.

30. Kumar MS, Miao ZH, Wyatt SK (2010) Influence of nutrient loads, feeding frequency and inoculum source on growth of Chlorrella vulgaris in digested piggy effluent culture medium. Bioresource Technology 101: 6012-6018.

31. Sahinkaya E, Dilek FB (2009) The growth behavior of Chlorrella vulgaris in the presence of 4-chlorophenol and 2, 4-dichlorophenol. Ecotoxicology and Environmental Safety 72: 781-786.

32. Saygidere SD, Okkay O (2008) Effect of 2, 4-dichlorophenoxyacetic acid on growth, protein and chlorophyll-a content of Chlorrella vulgaris and Spirulina platensis cells. Journal of Environmental Biology 29: 175.

33. Yen HW, Chen PW, Hsu CY, Lee L (2017) The use of autotrophic Chlorrella vulgaris in chromium (VI) reduction under different reduction conditions. Journal of the Taiwan Institute of Chemical Engineers 74: 1-6.

34. Wang R, Diao P, Chen Q, Wu H, Xu N, et al. (2017) Identification of novel pathways for biodegradation of bisphenol A by the green alga Desmodesmus sp. WRI1, combined with mechanistic analysis at the transcriptome level. Chemical Engineering Journal 321: 424-431.

35. Ji MK, Kabra AN, Choi J, Hwang JH, Kim JR, et al. (2014) Biodegradation of bisphenol A by the freshwater microalgae Chlamydomonas mexicana and Chlorrella vulgaris. Ecological Engineering 73: 260-269.

36. Gattullo CE, Bährs H, Steinberg CE, Loffredo E (2012) Removal of bisphenol A by the freshwater green alga Monoraphidium braunii and the role of natural organic matter. Science of the Total Environment 416: 501-516.

37. Dubey KK, Kumar S, Dixit D, Kumar P, Kumar D, et al. (2015) Implication of industrial waste for biomass and lipid production in Chlorrella minutissima under autotrophic, heterotrophic, and mixotrophic grown conditions. Applied Biochemistry and Biotechnology 176: 1581-1595.

38. Wang H, Guo S, Zheng B, Li C (2004) Growth and biochemical components of Chlorrella vulgaris under autotrophic heterotrophic and mixotrophic cultivations. Journal of South China University of Technology (Natural Science) 32: 47-50.

39. Van Wagener J, De Francisci D, Angelidaki I (2015) Comparison of mixotrophic to cyclic autotrophic/heterotrophic growth strategies to optimize productivity of Chlorrella sorokiniana. Journal of Applied Physiological 27: 1775-1782.

40. Ritchie RJ (2006) Consistent sets of spectrophotometric chlorophyll equations for acetone, methanol and ethanol solvents. Photosynthesis Research 89: 27-41.

41. Zheng H, Gao Z, Yin F, Ji X, Huang H (2012) Lipid production of Chlorrella vulgaris from lipid-extracted microalgal biomass residues through two-step enzymatic hydrolysis, Bioresource Technology 117: 1-6.

42. Beauchamp C, Fridovich I (1971) Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry 44: 276-287.

43. Lina SA, Raposo MF, Castro PM, Morais RM (2004) Biodegradation of p-chlorophenol by a microalgae consortium. Water Research 38: 97-102.

44. Chen Z, Song S, Wen Y, Zou Y, Liu H (2016) Toxicity of Cu (II) to the green alga Chlorrella vulgaris: a perspective of photosynthesis and oxidant stress. Environmental Science and Pollution Research 23: 17910-17918.

45. da Costa CH, Perreault F, Oukarroum A, Metegari SP, Popovic R, et al. (2016) Effect of chromium oxide (III) nanoparticles on the production of reactive oxygen species and photosystem II activity in the green alga Chlamydomonas reinhardtii. Science of the Total Environment 565: 951-960.

46. Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry 48: 909-930.

47. Ballesteros ML, Wunderlin DA, Bistoni MA (2009) Oxidative stress responses in different organs of Jenynsia multifilamenta exposed to endosulfan. Ecotoxicology and Environmental Safety 72: 199-205.

48. Ahscher RG, Erturk N, Heath LS (2002) Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. Journal of Experimental Botany 53: 1331-1341.

49. Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends in Plant Science 7: 405-410.

50. Chen X, Pei Y (2016) Effects of sodium pentaborate pentahydrate exposure on Chlorrella vulgaris growth, chlorophyll content, and enzyme activities. Ecotoxicology and Environmental Safety 132: 353-359.

51. Zhang W, Xiong B, Sun WF, An S, Lin KF, et al. (2014) Acute and chronic toxic effects of bisphenol A on Chlorrella pyrenoidosa and Scenedesmus obliquus. Environmental Toxicology 29: 714-722.