The influence of microplastics on the toxic effects and biodegradation of bisphenol A in the microalgae Chlorella pyrenoidosa

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Abstract  Bisphenol A (BPA) and polystyrene (PS) microplastics have attracted much attention because they are widely distributed in the environment, while their combined toxicity to aquatic organisms has rarely been studied. Therefore, this study explored the impact of microplastics on the toxic effects and biodegradation of BPA to the microalgae Chlorella pyrenoidosa. The results indicated that during the 16 days culture, PS (5 mg/L) increased the growth inhibition of BPA (1 mg/L and 10 mg/L) on C. pyrenoidosa compared to without PS. Similarly, PS (5 mg/L and 100 mg/L) also increased the degradation efficiency of BPA (1 mg/L and 10 mg/L) by algae. However, the changes of the chlorophyll content and the Fv/Fm value were opposite due to the hermetic and shading effect. Moreover, this study also found that five intermediates were formed during BPA degradation process because of the presence of oxidoreductase and glycosyltransferase. The results of the study provided vital information on the effect of PS on the toxicity and biodegradation of BPA to microalgal.

Keywords  Biodegradation · Bisphenol A · Chlorella pyrenoidosa · Microplastics

Introduction

Microplastics are defined as a particle size less than 5 mm, which is a new type of environmental pollutant with stable chemical properties and refractory to degradation (Horton et al. 2017; Lin et al. 2020). Microplastics can be discharged into the water environment in many ways, such as direct discharge of cosmetics or fine fibers into the water during industrial production and human activities (Herbort et al. 2018). Because of their low price and easy processing, plastics play a vital role in packaging, construction, transportation, electric power, medical devices and other fields and have been widely used in daily life (Yang et al. 2020b). Microplastics are widely distributed in water and exist in many forms. Polypropylene (PP), polyethylene (PE), polystyrene (PS), polyvinylchloride (PVC) and polyamide (PA) are the most common types of microplastics found in the freshwater systems, marine environments and sediments (Cincinelli et al. 2017; Klein et al. 2015).

Previous studies have shown that microplastics may have a variety of negative effects on the aquatic environment. For example, microplastics can reduce energy intake and affect the fecundity and offspring performance of aquatic organisms (Susarellu et al. 2016). Wu et al. investigated different
sizes (1 μm and 100 nm) of the PS microplastics on the effect of the algae growth, the results indicated that 1 μm microplastics show more adverse effects than 100 nm microplastics to Microcystis aeruginosa (Wu et al. 2021). Therefore, more research is needed to fully understand the impact of microplastics on different natural cycles, and an aspect that should be highlighted refers to the ability of microalgae to degrade organic pollutants.

Microalgae-based biotechnology has been considered as a promising alternative solution to current activated sludge systems to better treat nutrients and pharmaceutical contaminants (Xiong et al. 2017a). Phytoremediation by microalgae as a form of solar-powered decontamination is considered an environmentally friendly, sustainable, cost-effective reclamation strategy and technology (Xiong et al. 2016). The biodegradation of environmental organic pollutants by algae has also been reported by some researchers (Xie et al. 2020; Xiong et al. 2020), indicating that algae have the potential to remove pollutants in wastewater. Bisphenol A (BPA) (an endocrine disruptor compound) is used in the production of epoxy resin and polycarbonate (PC) plastics for various food and beverage packaging, baby bottles and dental sealants (Staples et al. 1998). In recent years, positive outcomes were obtained for the remediation of BPA-contaminated aqueous systems by green algae, including Monoraphidium braunii (Gattullo et al. 2012), Chlamydomonas Mexicana and Chlorella vulgaris (Ji et al. 2014) and Desmodesmus sp.WR1 (Wang et al. 2017). However, reports on the effect of microplastics on the biodegradation of BPA during the cultivation of microalgae are still lacking.

Chlorella pyrenoidosa is a freshwater green algae, which is sensitive to pollutants in the aquatic environment and is usually used as a model organism in ecotoxicity tests (Li et al. 2019, 2013; Liu et al. 2019; Zhao et al. 2017). Additionally, C. pyrenoidosa can accumulate and remove different pollutants in the aquatic environment (Peng et al. 2014; Wang et al. 2020, 2018). Therefore, in the present study, the ecotoxicological effects of different concentrations of BPA and PS microplastics on C. pyrenoidosa were evaluated according to the changes in microalgae growth. Moreover, further experiments were carried out to analyze the effects of PS on the biodegradation of BPA during microalgae cultivation. The kinetics of biodegradation of the BPA in combination and individually were also assessed.

Materials and methods

Chemicals and reagents

Bisphenol A (BPA) was purchased from Sigma-Aldrich (St. Louis, MO, USA) and was dissolved in methanol to obtain stock solutions. Polystyrene (PS) monodisperse microspheres (with the sizes of 9 μm in diameter) were provided by Big Goose (Tianjin) Technology Co., Ltd (Tianjin, China) with an initial concentration of 2.5% (W/V), and the stock suspension was prepared by diluting with ultrapure water. BG11 medium was supplied by Qingdao Hope Biotechnology Co., Ltd. (Qingdao, China). All of the chemical reagents used were analytical pure grade.

Algae cultivation

Microalgae C. pyrenoidosa were obtained from the Institute of Hydrobiology, the Chinese Academy of Science. The culture medium (BG11) and the materials used for algal cultures were autoclaved at 121 °C for 30 min before use. The algae cultivation was performed at 25 ± 1 °C under the illumination of 4000 lx with a 12/12 h light/dark cycle, the flasks were manually shaken three times in the incubator every day.

Growth inhibition test

The growth inhibition of BPA on C. pyrenoidosa was determined by monitoring the cell growth. The algae suspension, at its exponential phase, was inoculated into 250-mL flasks containing 100 mL of exposure medium at a density of 10^5 cells/mL. BPA treatment groups: BPA stock solutions were added in the algae suspension (1, 10, 20 and 30 mg/L) and measured at 2, 4, 6 and 8 days; BPA + PS treatment groups: PS solution was added in the algae suspension containing BPA (1 mg/L or 10 mg/L) to achieve the final concentration (5 mg/L or 100 mg/L) and measured at 2, 4, 6 and 8 days; BPA + PS treatment groups: PS solution was added in the algae suspension containing BPA (1 mg/L or 10 mg/L) to achieve the final concentration (5 mg/L or 100 mg/L) and measured at 6, 10 and 16 days; the groups without the PS and BPA algae suspension in the flasks served as the control groups. Cultivation conditions were as described above, the optical density of algal suspension was carried out at 680 nm (OD_{680}) by the Microplate Reader (Synergy
The algal concentration of *C. pyrenoidosa* was measured by using a blood cell counting plate under a light microscope (Ding et al. 2017). The growth inhibition rate of *C. pyrenoidosa* was based on a correlation between OD$_{680}$ and the microalgae density. The cell numbers of *C. pyrenoidosa* during the incubation were determined by the following equation:

$$\text{Algal cell numbers (cells/mL)} = 38.854 \times \text{OD}_{680} + 0.2124$$  \hspace{1cm} (1)

The growth inhibition rates were calculated as follows:

$$I\% = \frac{(C_0 - C)}{C_0}$$  \hspace{1cm} (2)

where $C_0$ is the cell numbers of the algal suspension of the control groups, $C$ is the algal cell numbers of treated groups and $I\%$ is the growth inhibition rate.

Analysis of photosynthetic pigment and chlorophyll fluorescence

Photosynthetic pigments, namely chlorophyll-a (Chl-a), chlorophyll-b (Chl-b) and total chlorophyll (Chl), were measured as described by Inskeep and Bloom (1985). The experimental design was as follows: 1 mg/L BPA, 1 mg/L BPA + 5 mg/L PS, 1 mg/L BPA + 100 mg/L PS, 10 mg/L BPA, 10 mg/L BPA + 5 mg/L PS and 10 mg/L BPA + 100 mg/L PS in the medium (containing 100 mL algae suspension) were conducted as treatment groups, the control groups were conducted with the absence of BPA and PS. After the 16 days of culture, a volume of 40 mL microalgae culture was centrifuged at 5000 rpm for 10 min. The supernatant was then decanted, and the substrate was then mixed with 80% acetone and incubated at 4 °C for 24 h in the dark. Next, the absorbance of the supernatant was measured at specified wavelengths of 470, 652.4 and 665.2 nm. Finally, the contents were computed using the following equations.

$$\text{Chl} - a (\text{mg/L}) = 12.63 \times A_{664.5} - 2.52 \times A_{647}$$  \hspace{1cm} (3)

$$\text{Chl} - b (\text{mg/L}) = 20.47 \times A_{647} - 4.73 \times A_{664.5}$$  \hspace{1cm} (4)

$$\text{Chl (mg/L)} = 17.95 \times A_{647} + 7.9 \times A_{664.5}$$  \hspace{1cm} (5)

The pulse amplitude modulation fluorometer AquaPen AP 110-C (photosystem instrument, Czech Republic) equipped with FluorPen 1.0 software was used to measure the photosynthetic activity of *C. pyrenoidosa*. Fluorescence parameters were calculated according to the method described by Wang et al. (Wang et al. 2011). Fv/Fm represents the maximal photochemical quantum yield and is a symbol for the light energy conversion efficiency of Photosystem II (PSII). Specifically, after the 16 days of cultivation, microalgae suspension (3 mL) was taken to measure the fluorescence intensity at 630 nm after avoided light for 0.5 h.

Analysis of BPA by HPLC

The experimental design was the same as part 2.4. At 0, 4, 8, 12 and 16 days after treatment, 2 mL of the algae suspension was collected and filtered by the polytetrafluoroethylene filter membrane. The concentration of BPA was monitored on a Dionex Ultimate 3000 HPLC). The HPLC system equipped with a binary pump with online solvent degasser, a diode array detector (DAD) and a TC-C18 column (Agilent, 5 μm, 250×4.6 mm). The temperature of the column was maintained at 35 °C, the mobile phase consisted of 15% methanol and 85% water with (0.2%) acetic acid (V: V) in a flow rate of 1.0 mL/min.

The removal kinetics of BPA were determined by the first-order model as follows:

$$\ln C = -kt + \ln C_0$$  \hspace{1cm} (6)

$$T_{1/2} = \ln 2/k$$  \hspace{1cm} (7)

where $C_0$ is the initial concentration of BPA at day 0, $C$ is the concentration of BPA at time $t$, $k$ and $t$ are the removal rate constant (day$^{-1}$) and removal period in days, respectively.

**Results and discussion**

Growth inhibition of PS and BPA to the microalgae

The relationship between cells density and the concentration of BPA, growth inhibition and BPA in the absence and presence of 5 mg/L PS was investigated. As shown in Fig. 1, this study found that both conditions have an adverse effect on the growth of the microalgae *C. pyrenoidosa*. Whether PS existed or
the algae density decreased with the increase of BPA concentration. For instance, compared with the control groups, in 1, 10, 20 and 30 mg/L BPA treatment, the growth inhibition rates were 12.52%, 12.85%, 21.75% and 41.85% at 8 days of exposure, respectively. These results were consistent with the research results reported by Ji et al., who found that the growth of microalgal *Chlorella vulgaris* was significantly inhibited by BPA at concentration of 50 mg/L at the same exposure time, and the bigger inhibition ratio of growth at 50 mg/L of BPA treatment was higher than that under the 25 mg/L and 10 mg/L BPA treatments in *Chlamydomonas mexicana* when exposed 7 days (Ji et al. 2014). A similar phenomenon was observed for the co-exposure of PS and BPA. For example, in 1 mg/L BPA, 1 mg/L BPA+5 mg/L PS, 10 mg/L BPA and 10 mg/L BPA+5 mg/L PS treatment, the growth inhibition rate were 7.27%, 9.52%, 13.48% and 16.66% at 16 days of exposure, respectively, which indicated that the co-exposure of PS and BPA had more toxic than treated with BPA alone. Similarly, Yi et al. reported that the presence of PS (0.55 μm) increased the toxicity of triphenyltin chloride (TPTCl) to *C. pyrenoidosa* (Yi et al. 2019). The explanation was given in 3.2.

Effects of BPA and PS on the *C. pyrenoidosa* photosystem

The photosynthetic pigment is responsible for absorbing light energy and elevating the pigment to a higher energy state, which is known as an excited state. Photosynthetic pigments can be used as an indicator of the photosynthetic capacity of algae (Lu et al. 2018). Changes in the pigmentation system have
been recognized as a defense mechanism under different types of stress conditions (Zhang et al. 2018). Common pigments in microalgae, such as chlorophyll (including chlorophyll a, chlorophyll b and total chlorophyll) and carotenoids, are usually considered to be indicators of cell adaptation. Chlorophylls absorb light or transfer light energy into photosystem reaction center, or convert light energy to electoral energy, thereby playing essential roles in photosynthesis (Yang et al. 2020a). As observed in Table 1, compared with the control groups, the content of chlorophyll-a (Chl-a), chlorophyll-b (Chl-b) and total chlorophyll (Chl) in the cells increased after treatment for 16 days. As the PS concentration increased, the C. pyrenoidosa chlorophyll content decreased, and the low concentration of BPA (1 mg/L BPA) with 5 mg/L PS had the highest chlorophyll content. Although a decrease in chlorophyll content is common under stress-induced conditions, there are reports that when treated with low concentrations of pollutants, the chlorophyll content of C. pyrenoidosa increased and decreased at higher doses. Wan et al. show that when exposed to low concentrations of levofloxacin, the chlorophyll content in Microcystis flosaquae increased significantly, while the high concentration of levofloxacin reduced the chlorophyll content, which is due to the hermetic effect caused by antibiotic stress (Wan et al. 2014). A similar phenomenon was also observed in microalgae Scenedesmus obliquus when exposed to ofloxacin, the content of chlorophyll-a, chlorophyll-b and chlorophyll, in the cells initially increased when the medium’s ofloxacin was 10 mg/L (Yang et al. 2020a).

Fv/Fm indicates photosynthetic activity, which is widely used as an important indicator for monitoring algal photosystem II (PSII) activity (Yang et al. 2020b). As shown in Fig. 2, the Fv/Fm values of C. pyrenoidosa in the single BPA and BPA combined PS treatment at different concentrations were lower than that of the control groups after 16 days, indicating that the PS had a negative effect on the PSII activity of C. pyrenoidosa. This negative influence was increased with concentration of the PS. The Fv/Fm values decreased by 0.2%, 5.1%, 12.1%, 6.9%, 9.5% and 15.6% for 1 mg/L BPA, 1 mg/L BPA + 5 mg/L PS, 1 mg/L BPA + 100 mg/L PS, 10 mg/L BPA, 10 mg/L BPA + 5 mg/L PS and 10 mg/L BPA + 100 mg/L PS, respectively. Overall, the above obtained Fv/Fm values of C. pyrenoidosa in 100 mg/L PS microplastics treatments were lower than those in the 5 mg/L PS microplastics treatments for both 1 mg/L and 10 mg/L of BPA exposure. This phenomenon can be attributed to the shading effect. A similar result was reported by Zhang et al. that PVC had significantly negative effects on chlorophyll content and PSII activity of microalgae Skeletonema costatum (Zhang et al. 2017). Mao et al. also obtained the conclusion that the bigger inhibition ratio of Fv/Fm at 100 mg/L PS microplastics (0.1 mm) treatment was higher than that under the 10 mg/L and 50 mg/L PS microplastics (0.1 mm) treatments (Mao et al. 2018).

Effect of PS microplastics on the C. pyrenoidosa removal of BPA

Due to its endogenous catabolic system, heterotrophic capacity and role in carbon fixation and turnover, microalgae have the potential to remove organic pollutants (Xiong et al. 2017b). The removal kinetics of BPA by C. pyrenoidosa are shown in Fig. 3, after incubation for 16 days, the removal efficiencies of BPA were 23.76%, 39.65%, 31.80%, 34.94, 45.53% and 42.11% by incubation with this microalgae cells for 1 mg/L BPA, 1 mg/L BPA + 5 mg/L PS, 1 mg/L BPA + 100 mg/L PS, 10 mg/L BPA, 10 mg/L BPA + 5 mg/L PS and 10 mg/L BPA + 100 mg/L PS, respectively. A higher removal rate of BPA was observed at a lower exposure level with PS.

| Table 1 Photosynthetic activity of C. pyrenoidosa under different conditions at the 16th day of culture |
|--------------------------------------------------|--------------------------------------------------|--------------------------------------------------|--------------------------------------------------|--------------------------------------------------|--------------------------------------------------|--------------------------------------------------|--------------------------------------------------|
| Treatment                  | 1 mg/L BPA                                      | 1 mg/L BPA + 5 mg/L PS                           | 1 mg/L BPA + 100 mg/L PS                          | 10 mg/L BPA                                      | 10 mg/L BPA + 5 mg/L PS                           | 10 mg/L BPA + 100 mg/L PS                         | Control                                           |
| Chl-a (mg/L)               | 37.96                                          | 48.98                                          | 36.58                                           | 43.43                                          | 43.48                                          | 37.49                                           | 36.01                                              |
| Chl-b (mg/L)               | 20.62                                          | 28.01                                          | 21.54                                           | 22.37                                          | 22.97                                          | 20.87                                           | 20.26                                              |
| Chl (mg/L)                 | 58.58                                          | 76.99                                          | 58.12                                           | 65.80                                          | 66.45                                          | 58.36                                           | 56.27                                              |
**Fig. 2** The photosynthetic indices Fv/Fm of *C. pyrenoidosa* when exposed to BPA and PS microplastics for 16 days.

**Fig. 3** Effect of PS on the removal of BPA by incubation with *C. pyrenoidosa*.
Compared with the treatment without PS (exposure to 1 mg/L BPA and 10 mg/L BPA), the BPA removal efficiency of lower PS exposure level (5 mg/L) increased by 15.89% and 10.59%, respectively, suggesting that PS can accelerate the removal of BPA in the algae medium. These results are consistent with previous reports that the co-presence of polystyrene nanoplastic can enhance the degradation of ibuprofen in the medium of *C. pyrenoidosa*.

The kinetic analysis showed that the kinetic removal rate constant (k, day\(^{-1}\)) of 1 mg/L BPA, 1 mg/L BPA + 5 mg/L PS, 1 mg/L BPA + 100 mg/L PS, 10 mg/L BPA, 10 mg/L BPA + 5 mg/L PS and 10 mg/L BPA + 100 mg/L PS ranged from 0.0149 to 0.0374 day\(^{-1}\), and the degradation half-life (T\(_{1/2}\), day) was calculated to be 18.53–46.52 days (Table 2).

Furthermore, the removal of BPA via abiotic factors was evaluated through the addition of BPA to the medium without any *C. pyrenoidosa* inoculation. The initial concentration of BPA in the abiotic control did not show any change throughout 16 days. Thus, microalgae biomass is the main factor in the removal of BPA in this study, and the contribution of abiotic factors in the removal of BPA is negligible.

**Table 2** Kinetic parameters of BPA degradation during 16 days of cultivation

| Species | 1 mg/L BPA | 1 mg/L BPA + 5 mg/L PS | 1 mg/L BPA + 100 mg/L PS | 10 mg/L BPA | 10 mg/L BPA + 5 mg/L PS | 10 mg/L BPA + 100 mg/L PS |
|---------|------------|------------------------|--------------------------|------------|------------------------|--------------------------|
| k (day\(^{-1}\)) | 0.0149 | 0.0297 | 0.0199 | 0.0248 | 0.0374 | 0.031 |
| T\(_{1/2}\) (day) | 46.52 | 23.34 | 34.83 | 27.95 | 18.53 | 22.36 |
| R\(^2\) | 0.9078 | 0.9058 | 0.8165 | 0.8871 | 0.8384 | 0.8544 |
| Total removal (%) | 23.76 | 39.65 | 31.80 | 34.94 | 45.53 | 42.11 |

k, kinetic removal rate constant (day\(^{-1}\)); T\(_{1/2}\), removal half-life (day); R\(^2\), correlation coefficient

As can be seen from Fig. 4, the peak area of BPA decreased with the extension of the incubation time, and some new peaks with retention times different from that of peak-BPA were appeared after 4 days,
indicating that some intermediates were formed during the \textit{C. pyrenoidosa} degradation of BPA. Potential transformation products of BPA were identified based on the mass-to-charge ratios (m/z), and their chemical structures were confirmed by the characteristic fragment ions. The mass spectra information after microalgal degradation is shown in Fig. S1. Based on these results and previous literature (Wang et al. 2017), the transformation pathways for BPA are proposed in Fig. 5. P1 was formed owing to oxidoreductase activity, and P1 further lost a molecule of H$_2$O leading to the formation of P2. García-Rodríguez et al. revealed that phenolic compounds could be oxidized by oxidoreductases such as polyphenol oxidase and peroxidase (García-Rodríguez et al. 2015). In addition, oxidoreductase can catalyze the oxidative degradation of phenolic compounds (Taticchi et al. 2013). Therefore, our present study indicated that \textit{C. pyrenoidosa} over-expressed oxidoreductase encoding genes to promote BPA oxidation to form hydroxyl groups on the benzene ring of BPA. P3 could be formed through another pathway via the action of oxidoreductase, indicating that the addition of a hydroxyl groups to the P3. With the subsequent glycosyltransferase, P4 was further formed, a similar result was obtained for the biodegradation of BPA by the green alga \textit{Desmodesmus} sp.WR1 (Wang et al. 2017). Hyung Ko et al. (2006) demonstrated that glycosyltransferase was able to glycosylate phenolics (Hyung Ko et al. 2006). In 2003, Morohoshi et al. showed that BPA glycosylation resulted in the inability to detect the level of estrogenic activity in the yeast two-hybrid assay (Morohoshi et al. 2003). Therefore, our results also suggested that \textit{C. pyrenoidosa} was able to transform BPA into non-toxic metabolites via glycosylation. P5 resulted from the cleavage of -CH$_2$OH, and the fragment m/z 406 (P6) corresponded to the subsequent loss of a hydroxyl group.

**Conclusions**

The effects of PS on the toxic effects and biodegradation and removal of BPA in \textit{C. pyrenoidosa} were reported in this study. In conclusion, the co-exposure of PS and BPA increased the growth inhibitory effect, decreased the chlorophyll content and enhanced the removal efficiency of BPA in the medium. In addition, five intermediates were found based on the mass-to-charge ratios (m/z) in the biodegradation process. The proposed transformation pathways of BPA by \textit{C. pyrenoidosa} are shown in Fig. 5.
process. According to this, the degradation pathway of BPA by *C. pyrenoidosa* was proposed in the presence of PS. More studies should be needed to explore the toxicity of microplastics and contaminants in the aquatic system to gain a better understanding of the detoxification mechanism behind the interactions of aquatic organisms.

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