Science of the Total Environment 661 (2019) 55–62

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Phycoremediation of coastal waters contaminated with bisphenol A by green tidal algae *Ulva prolifera*

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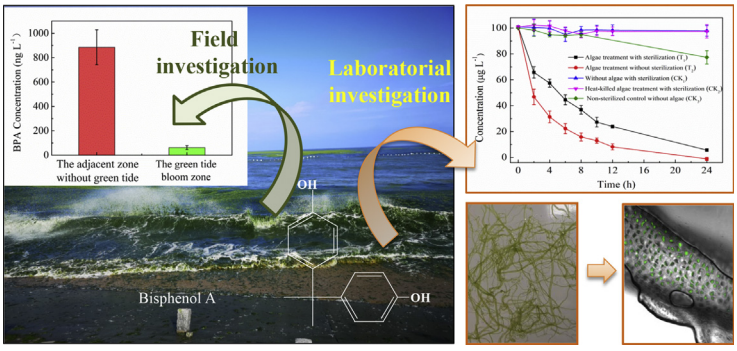
HIGHLIGHTS

- New phycoremediation of coastal water with BPA occurred during green tide bloom.
- >94% of BPA was removed by the green-tidal algae *U. prolifera*.
- BPA removal efficiency has positive relationship with light, nutrient and temperature.
- High removal efficiency (>94%) was achieved at environmental relevant concentrations.
- BPA concentration in green tide bloom area was much lower than that in adjacent area.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 24 October 2018
Received in revised form 12 January 2019
Accepted 12 January 2019
Available online 15 January 2019

Editor: Yolanda Picó

Keywords:
Phycoremediation
Ulva prolifera
Green tide
Phenolic endocrine-disrupting chemicals
Bisphenol A
Coastal waters

ABSTRACT

The phycoremediation of coastal water contaminated with bisphenol A (BPA) by *Ulva prolifera* (*U. prolifera*) during green tide blooming was investigated. The results demonstrated that BPA could be removed rapidly in the presence of *U. prolifera*. >94.3% of BPA was removed by live *U. prolifera* while <2.5% of BPA was removed by dead biomass. The accumulation of BPA in *U. prolifera* was confirmed by laser confocal scanning microscopy (LCSM). Uptake experiments under different conditions showed that the removal efficiency of BPA by *U. prolifera* had positive relationships with light, nutrient and temperature while the salinity had no effect. A linear relationship existed between the removal efficiency and the BPA initial concentration when the BPA initial concentration increased from 50 to1000 μg L^{-1}, indicating the high tolerance of the green-tidal algae to the toxic effect of BPA. High BPA removal efficiency (>94%) was achieved at the environmental relevant concentrations of BPA. The field investigation indicated that the BPA concentration in the coastal water in the green tide blooming area was much lower than that in the adjacent coastal water without green tide. The contribution of the green-tidal algae in the removal of BPA in the coastal waters was remarkable due to the high BPA removal efficiency, and high biomass & huge covered area of the *U. prolifera* during the outbreak of green tide. These findings demonstrate a new important phycoremediation process for coastal water containing typical endocrine-disrupting chemicals (EDCs) during the green tide blooming.

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https://doi.org/10.1016/j.scitotenv.2019.01.132
0048-9697/

1. Introduction

Green tides are becoming a global environmental issue to attract public attention due to the excessive nutrients anthropogenically derived in coastal waters (Keesing et al., 2011; Wei et al., 2018). China experienced green tides in the Yellow Sea consecutively from 2007 to 2018. In every wet season of these years, the free-floating algal patches of green tides started from the coastal area of Jiangsu Province, then moved northward progressively under wind-current action, and finally destined to the southern coastal zone of Shandong Peninsula with masses of algae being washed ashore, accompanying with various adverse impacts on indigenous biodiversity, aquaculture and tourism (Liu et al., 2015; Luo et al., 2012; Van Alstyne et al., 2015). *Ulva prolifera* (*U. prolifera*) is considered as the dominant species in green tides (Zhao et al., 2015). Its filamentous characteristics provide them a higher ratio of surface to volume (S/V), which has been confirmed to be feasible for uptake of nutrient (Fan et al., 2014; Luo et al., 2012; Xu et al., 2016), metals (Özer et al., 2009), and polycyclic aromatic hydrocarbons (PAHs) (C. Zhang et al., 2017). However, there has been rare information on its contribution to the fate and transport of emerging contaminants in coastal and marine environments.

Endocrine-disrupting chemicals (EDCs) are a kind of emerging contaminants that could disturb the normal hormone activities of humans and animals (Abargues et al., 2018; Wang et al., 2013), causing negative effects on their behavior, growth and immune function. Importantly, these endocrine disruptor effects could take place even at a very low concentration (Wang et al., 2013; M. Zhang et al., 2017). Anthropogenic inputs, active industrialization and an increase in the plastics usage are increasing the potential threat of EDCs to coastal environments (Xu et al., 2014). Bisphenol A (BPA), a common industrial raw material, has been widely used for the production of polycarbonate plastics and epoxy resins (Chiu et al., 2018). As a typical representative EDCs, BPA is a major contributor to the endocrine-disrupting effects in aquatic environments (Eio et al., 2015; Xu et al., 2014). In the global aquatic ecosystems, except for acting as a primary producer (Nakajima et al., 2007), algae are also considered to play an important role in the removal of hydrophobic organic contaminants (Guo et al., 2017). Our previous work assessed the uptake removal of phenanthrene by *U. prolifera* that demonstrated its potential on the removal of hydrophobic organic contaminants (C. Zhang et al., 2017). The octanol-water partition coefficient (K_{ow}) for BPA is 3.40, which means it may be accumulated by algal because of its moderate hydrophobic property (Isobe et al., 2007). Microalgae have been reported to play important role in the fate of EDCs in freshwater aquatic environments (Gattullo et al., 2012; Guo et al., 2017; Hom-Diaz et al., 2015; Nakajima et al., 2007; Ruksrithong and Phattarapattamawong, 2017; Wang et al., 2017). Gattullo et al. (2012) studied the removal of BPA by the microalga *Monoraphidium braunii* to find that approximately 35%–48% of BPA (2–10 mg L^{-1}) was removed from wastewater and BPA at the lower concentrations (<4 mg L^{-1}) was not toxic for alga. In addition, natural organic matter at any concentration scarcely influenced the BPA removal. Wang et al. (2017) reported that green microalga *Desmodesmus* sp. WR1 isolated from municipal wastewater was able to remove BPA (1, 3, 5.5, 13.5 mg L^{-1}) with the removal rate ranging from 18% to 57% and BPA was transformed into two products. Rare information is available on the role of macroalgae, especially the harmful algae which are of such huge biomass at a given period, in the fate of BPA in costal water.

The primary goal of our study is to evaluate the potential removal of BPA from coastal water by *Ulva prolifera*. Since the *U. prolifera* is of high tolerance on the fluctuations in temperature, light and salinity in coastal zones (Luo et al., 2012), the influence of various environmental factors on the removal of BPA by *U. prolifera* is also evaluated. This information is important for understanding the natural attenuation of EDCs in coastal water during the green tide blooming, which would then be beneficial to clarify the exposure routes and assess the ecological risks related to these hydrophobic organic contaminants.

2. Materials and methods

2.1. Chemicals and materials

BPA (purity > 99%) was supplied by Sigma-Aldrich (St. Louis MO, USA). The internal standard (phenanthrene-d_{10}, GC–MS analysis) obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). The *N*,*O*-bis (trimethylsilyl) trifluoroacetamide (BSTFA, purity > 98%) and trimethylchlorosilane (TMCS, purity > 98%) were purchased from Alfa Aesar (Ward Hill, MA). HPLC grade solvents including methanol, acetonitrile and dichloromethane were purchased from Merck (Germany). The sodium hydrogen phosphate, anhydrous sodium sulfate, sodium hydroxide, hydrochloric acid, and sodium nitrate were all of analytical reagents. The anhydrous sodium sulfate was put into in a muffle furnace and dried at 450 °C for 4 h before usage. To prepare the stock solutions of BPA (1 g L^{-1}), an appropriate amount of BPA was dissolved in methanol in a volumetric flask and stored at −20 °C.

2.2. The U. prolifera and seawater for experiments

Free-floating *U. prolifera* was collected in June 21, 2018 from the coastal water of the beach of Rushan city, China. There was a severe outbreak of green tide in this coastal area. In order to slow down the aging process of *Ulva prolifera*, the algae were incubated in the glass tank with natural seawater at 10 °C with a 12 h/12 h dark/light cycle in a GXZ-380B-LED temperature-controlled incubator (Ningbo, China) after washing several times using sterile seawater. The irradiance was set at 100 μmol photons m^{-2} s^{-1}. Before conducting the experiments, the algae were transferred to an incubator with the experimental temperature for 48 h. The incubation media were refreshed every 7 days. Natural seawater (salinity: 32 g L^{-1}, pH: 7.84–8.09), collected from the beach of Yantai, China, was filtered through Whatman GF/C glass fiber papers (0.7 μm pore size) and used in all experiments of this study.

2.3. BPA uptake experiments

The uptake removal efficiency of BPA from the solution by live *U. prolifera* under different experimental assays was evaluated in beaker (500 mL) with 400 mL of sterilized seawater containing relevant concentrations of BPA and the algae. Each beaker was sealed to avoid the evaporation loss of BPA. For the live algae treatment (T_1), the algae (4.0 g L^{-1}) were added into the beaker. For the heat-killed algae treatment (CK_2), algae with the same amount of the live algae treatment were autoclaved at 121 °C for 20 min before used for the experiment. The beakers without algae biomass (CK_1) were prepared as the abiotic controls to monitor abiotic loss of BPA due to volatilization and photodegradation. A biotic treatment (T_2) by adding algae and BPA to natural seawater without sterilization and a non-sterilized control without algae treatment (CK_3) were also conducted to discuss the effect of microorganisms. The algal epiphyte was obtained by transferring 1.6 g alga samples into a sterile 50 mL polyethylene bottle containing 30 mL of 0.01 mol L^{-1} PBS solution (pH 7.2–7.4). After shaking for 30 min at 200 rpm, the samples were centrifuged at 4500 rpm for 10 min to collect its supernatant. Finally, all the algal epiphyte was washed by filtered seawater without sterilization into beaker for CK_3 experiments. The initial concentration of BPA (100 μg L^{-1}) was set through diluting the BPA stock solution (1 g L^{-1}) with the sterile seawater. The water and algal samples were sampled at set time intervals. The residential BPA concentration in both seawater medium and algal biomass was detected by ultra high performance liquid chromatography (UHPLC) and gas chromatography–mass spectrometry (GC–MS), respectively.

Different experimental setups were also conducted to observe the influence of physicochemical variables such as photoperiod (0:24, 12:12 and 24:0 (light:darkness)), salinity (16, 24 and 32 g L^{-1}), temperature (10, 20 and 30 °C), and nutrient deficiency (0 μM NO_3^-/0 μM PO_4^{2-}, 50 μM NO_3^-/5 μM PO_4^{2-} and 150 μM NO_3^-/15 μM PO_4^{2-}) on the removal

of BPA in the presence of live green-tidal algae. In order to evaluate the influence of different photoperiod on the removal, the algae were put into darkness, a photoperiod of 12:12 (light:darkness), and a photoperiod of 24:0 (light:darkness), respectively. To investigate BPA removal under various salinities, the water salinity was set as 16, 24, and 32 g L^{-1}. The lower salinity water was obtained through diluting seawater (salinity of 32 g L^{-1}) with corresponding volume of ultra-purified water. To evaluate the influence of nutrient, the nutrient concentration gradients (0 μM (NO_3^-)/0 μM (PO_4^{2-}), 50 μM (NO_3^-)/5 μM (PO_4^{2-}) and 150 μM (NO_3^-)/15 μM (PO_4^{2-})) were prepared. Temperature was set to 10 °C, 20 °C and 30 °C using temperature-controlled incubator to observe the removal of BPA by live *U. prolifera*. Additionally, the effect of different initial BPA concentrations, obtained through diluting the BPA stock solution with sterile sea water to 50, 100, 200, 500, and 1000 μg L^{-1}, on removal rate was also evaluated by keeping the mass of *U. prolifera* as 4 g L^{-1}. The final BPA concentration was measured using UHPLC. None of the environmental factors tested significantly inhibited growth of *U. prolifera*.

2.4. Field study

The field studies were performed in coastal area, where there was green tide blooming area as we collected fresh *U. prolifera* (U_1, U_2, and U_3). Additionally, the water samples (C_1, C_2, and C_3) were also collected as control from the adjacent area without green tide, 2 km away from the green tide blooming zone. The area of each site is approximately 67,000 m^2. The two sampling locations in Rushan city were chosen to analyze the concentration of BPA in seawater for evaluating the phycoremediation of the coastal water polluted with BPA through the green tide blooming. Surface water samples, 0.3 m below the air–water interface, were collected with 1 L pre-cleaned amber glass sample bottles. All of the samples were quickly transported to the laboratory for BPA analysis. Physical and chemical properties of coastal water in these two study areas were also measured. The salinity, pH, and turbidity of coastal water from the green tide blooming zone were 31 g L^{-1}, 8.03, and 20.92 NTU, respectively. The salinity, pH, and turbidity of coastal water from the adjacent zone without green tide were 31 g L^{-1}, 7.96 and 39.52 NTU, respectively.

2.5. Analytical methods

The aqueous sample from the BPA removal experiments was immediately centrifuged at 12,000 rpm for 6 min. The residual concentrations of BPA in water samples were analyzed using a Waters ACQUITY UHPLC system (Milford, USA). The UHPLC system was equipped with a C18 reverse phase column (2.1 × 50 mm, 1.7 μm). The mobile phase was H_2O/methanol (40/60, v/v %) while the flow rate was set at 0.2 mL min^{-1}. The injection volume was 10 μL, and fluorescence of the eluted compounds was monitored at 305 nm with excitation at 225 nm by a Waters fluorescence detector (Milford, USA).

In order to monitor the BPA concentration in algae samples, the extraction procedures were conducted. Fresh algae (1.6 g) were homogenized with 5 μL internal standard solution (phenanthrene-d_{10}, 100 mg L^{-1}). A 2 g of anhydrous sodium sulfate was next added to remove water from the samples, followed by the addition of 5 g of diatomaceous earth to improve the permeability. The samples were then extracted by an APLE-1000 accelerated solvent extraction apparatus (Titan, Beijing, China) equipped with 22 mL extraction cells (stainless-steel). The samples were extracted with absolute ethanol under the following conditions: preheating for 2 min, heating for 5 min, extraction at 10.0 MPa and 120 °C, static extraction for 3 min, two extraction cycles, purge volume of 30% and purge time of 1.5 min. The extracted liquid was concentrated until nearly dry by nitrogen evaporator, then reconstituted with 100 μL dichloromethane (CH_2Cl_2) and derivatized with 50 μL BSTFA containing 1% TMCS at 70 °C for 2 h prior to GC–MS analysis.

For BPA analysis, the environmental samples were filtered using glass fiber papers, and then adjusted pH with hydrochloric acid to 3.0. These samples were spiked with a 10 μL of phenanthrene-d_{10} (5 mg L^{-1}) before the BPA was extracted from the 0.5 L water samples with liquid-liquid extraction (LLE). It is theoretically good to use isotope-labeled BPA as the internal standard for BPA analysis. However, phenanthrene-d_{10} showed higher recovery rate than isotope-labeled BPA (BPA-d_{16}) in the pre-tests due to closer retention time between BPA-d_{16} and BPA by GC/MS analytical methods used in this study, which dissatisfied the detection requirements. Therefore, phenanthrene-d_{10} not isotope-labeled BPA was chosen as the internal standard for BPA analysis. The extraction treatment was conducted with 30 mL of CH_2Cl_2 for twice. The total of 60 mL of CH_2Cl_2 was concentrated to about 1.5 mL in a water bath (70 °C), then passed through anhydrous sodium sulfate to remove water. The solution is concentrated to nearly dry using nitrogen evaporator, and redissolved in 100 μL CH_2Cl_2 and derivatized with 50 μL BSTFA (containing 1% TMCS) at 70 °C for 2 h prior to GC–MS analysis.

The extraction of the algal samples and environmental samples were analyzed using an Agilent 7820A GC system (Palo Alto, CA, USA) coupled to a M7 single quadrupole MS system (Persee Co., Beijing, China), equipping with a 30 m DB-5MS column (0.25 mm I.D., Agilent J&W Scientific, Palo Alto, CA, USA). The extract sample with volume of 1 μL was injected in pulsed splitless mode (12 psi for 0.75 min) with helium (purity > 99.999%, 1.0 mL min^{-1}) as a carrier gas. The temperature program for column oven was set at 100 °C for 1 min, then increased to 220 °C at a 20 °C min^{-1} rate and kept for 2 min, continually increased to 290 °C at 35 °C min^{-1} rate, and kept for 5 min. The total runtime was 16.00 min, including a solvent delay time of 7 min. The temperatures of the injector, transfer line and ion source were held at 280 °C, 280 °C and 230 °C, respectively. The confirmation and identification of BPA and phenanthren-d_{10} in the extract were based on their retention time and selected ion. The quantitative and qualitative ions of the compounds for selected ion monitoring (SIM) mode operation were *m/z* 357, 358, and 372 for BPA (2TMS derivative), and *m/z* 187, 188, and 189 for phenanthren-d_{10}. The method recoveries ranged from 81.3% to 94.9% while the RSD values were in the range of 2.7%–8.6% to meet the detection requirements.

The *in situ* observation was conducted to visualize the locations of BPA in/on live and heat-killed algae with an Olympus Fluoview FV1000 laser scanning confocal microscope (LCSM) (40× objective lens). The LCSM wavelength was 405 nm. Images of BPA in/on the algae tissues were visually inspected and processed by the Viewer software (Olympus Fluoview Ver.2.1c). All selected algal samples were recorded with three images under brightfield image, green fluorescence image and overlay image.

2.6. Statistical analysis and data calculation

All experiments were conducted in triplicate. The results were processed using Origin 8.5 and IBM SPSS 20.0. One-way ANOVA and Tukey's multiple comparisons were applied to compare the differences of the BPA removal efficiency among various treatments. Statistical significance was considered as $p < 0.05$.

In this study, we assumed that the removal of BPA in the algal system was achieved by BPA uptake, and the first-order kinetic (Eq. (1)) was used to describe the removal rate of BPA:

$$\ln C_t = \ln C_0 - kt \quad (1)$$

where C_0 (μg L^{-1}) and C_t (μg L^{-1}) are the initial BPA concentration and residential BPA concentration at time t (hours) in the aqueous solution, respectively; k (h^{-1}) is the first-order degradation rate constant which is obtained as the slope of the linear regression to the BPA removal data points.

3. Results and discussion

3.1. The removal of BPA in the presence of U. prolifera

A representative time course for the concentration of BPA remained in seawater in the presence of the green-tidal algae, the *U. prolifera*, was presented in Fig. 1. The residual concentrations of BPA in the sterilized medium without algae (CK_1) kept constant at 100 μg L^{-1} during the 24 h exposure, indicating that abiotic loss due to volatilization and photodegradation was negligible. While in the presence of *U. prolifera* (T_1), the concentration of BPA in the medium declined faster from 100.00 μg L^{-1} to 5.69 μg L^{-1} in 24 h, corresponding to removal efficiency of 94.3%. The difference of removal efficiency between CK_1 and T_1 manifested that the BPA removal was due to the engagement of *U. prolifera*. Simultaneously, the BPA removal efficiency in the algae-added medium without sterilization (T_2) was 100% which was slightly higher than that of T_1 treatment, indicating that bacteria could enhance removal efficiency of BPA by *U. prolifera* to some extent. Therefore, a non-sterilized control without algae treatment (CK_3) standing for the microbial biodegradation process without algae during BPA removal process was added to verify this hypothesis. However, only removal efficiency of 22.59% was obtained during the 24 h incubation, which confirmed that the BPA was mainly removed through green-tidal algae uptake rather than the microbial biodegradation. Compared to BPA removal in the T_1 treatment, the residual concentrations of BPA in the heat-killed *U. prolifera* (CK_2) with sterilization also showed no significant changes during the 24 h exposure, demonstrating that most of the BPA was removed due to the engagement of live *U. prolifera* (uptake process) rather than the adsorption process. These results were in accordance with Eio et al. (2015), who found that BPA adsorption by *Chlorella sorokiniana* were <1%, and the BPA was removed efficiently in algal (*Chlorella sorokiniana*)-activated sludge bacterial system from 10 mg L^{-1} to below the detection limit, while that in the monoculture of algal system was 38.5%. Wang et al. (2017) performed similar experiments with green microalga *Desmodesmus* sp. WR1 and achieved removal efficiency of 54% under the lowest initial BPA concentration (1 mg L^{-1}) in a 10-day treatment.

In order to know the removal behaviors of BPA in the presence of *U. prolifera* better, the removal kinetics was investigated. As it was fitted by Eq. (1), the removal data of algal treatment (T_1) and algal-bacterial treatment (T_2) were both fitted first-order kinetic model well (R^2 > 0.995). The first-order degradation rate constant (k) in T_1 was 0.116 h^{-1}. This value was higher than the rate constant k (0.039 h^{-1}) determined by Guo et al. (2017), who explored the bioaccumulation and elimination process of ^{14}C-labeled BPA by *Chlorella pyrenoidosa* (freshwater green microalgae) which showed a lower accumulated rate (15%) after ten days of 10.8 μg BPA L^{-1} exposure. The difference in the pollution acclimation ability of different algae species might lead to the difference in the accumulation of BPA by algae (Ike et al., 2000).

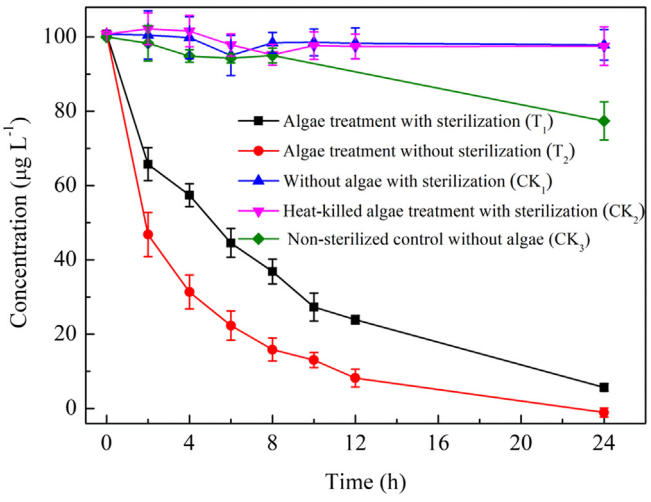

Fig. 1. Changes of bisphenol A concentrations in aqueous medium during incubation.

The EDCs could undergo many natural attenuation processes such as photolysis, adsorption, biodegradation, chemical oxidation and volatilization (Koumaki et al., 2018). The high removal efficiency of BPA in the presence of the green-tidal algae, the *U. prolifera*, suggested a potential new natural phycoremediation of the coastal waters contaminated with EDCs during the green tide blooming. Additionally, LCSM which is a real-time tool for observing organic pollutant within plant directly (Wang et al., 2012) was applied for the *in situ* monitoring of the removal process of BPA. As it was shown in Fig. 2, BPA fluorescence was clearly observed in live *U. prolifera* rather than in heat-killed *U. prolifera*, which further confirmed the engagement of live *U. prolifera* in BPA removal and it was the algal accumulation but not the adsorption process that acted as the main removal process in the presence of *U. prolifera*.

The contents of BPA detected in live (T_1) and heat-killed algae (CK_2) were 9.4 μg g^{-1} and below the detection limit after 24 h, respectively, confirming the engagement of algae in BPA removal. According to the mass balance studies, only 37.6% of the total amount of BPA was obtained from the algae biomass of the live algae (treatment T_1), which was much lower than the BPA removal efficiency (94.3%) during the incubation. The rational explanation for the low BPA recovery from algae biomass was that the absorbed BPA was subsequently metabolized in the algae, since two new peaks with retention times differed from that of peak-BPA were observed in HPLC spectra (data not shown). According to Nakajima et al. (2007), BPA could be transformed to its glycosides which are non- or less-toxic for green microalgae. Eio et al. (2015) used GC–MS to detect two degradation intermediates including hydroxyl-BPA (OH-BPA) and p-hydroquinone in the monoculture of algal system.

3.2. Effect of the photoperiod and salinity on BPA removal

During the outbreak of green tide, *U. prolifera* formed unequal dense mats in the intertidal zones, where the algae must experience different photoperiod, such as contrasting light environments in open sites and relatively dim light because of layer coverage. According to Fig. 3(a), light irradiation had an important role in the removal of BPA. During the 24 h exposure with different photoperiod, the removal efficiency under complete darkness condition, photoperiod 12:12 (light:darkness) condition, and photoperiod 24:0 (light:darkness) condition were 41.8%, 94.3%, and 98.5%, respectively. Compared with light-irradiated treatments, the complete darkness treatment showed lower removal efficiency, confirming that BPA removal efficiency was influenced by light irradiation to some degrees. According to Luo et al. (2014), the treatments under the illumination of golden and white light had higher benzo[*a*]pyrene removal efficiency by microalgae (*S. capricornutum*) compared with treatment in complete darkness. Algae use light for the photosynthesis which convert light energy into chemical energy used for further metabolic activities (Singh and Singh, 2015). The complete darkness could inhibit the metabolism of *U. prolifera*, which subsequently led to the inhibition of BPA removal. The removal of BPA under the 12:12 (light:darkness) photoperiod condition was similar to that under the 24:0 (light:darkness) photoperiod condition, indicating that the removal of BPA was not significantly influenced by the illumination duration.

The influence of salinity at three levels (16, 24, and 32 g L^{-1}) on BPA removal was also evaluated due to the salinity fluctuations in the coastal waters. As it is shown in Fig. 3(b), the BPA removal rate was found to be similar as the salinity increased from 16 g L^{-1} to 32 g L^{-1}. Results from One-way ANOVA and Tukey's multiple

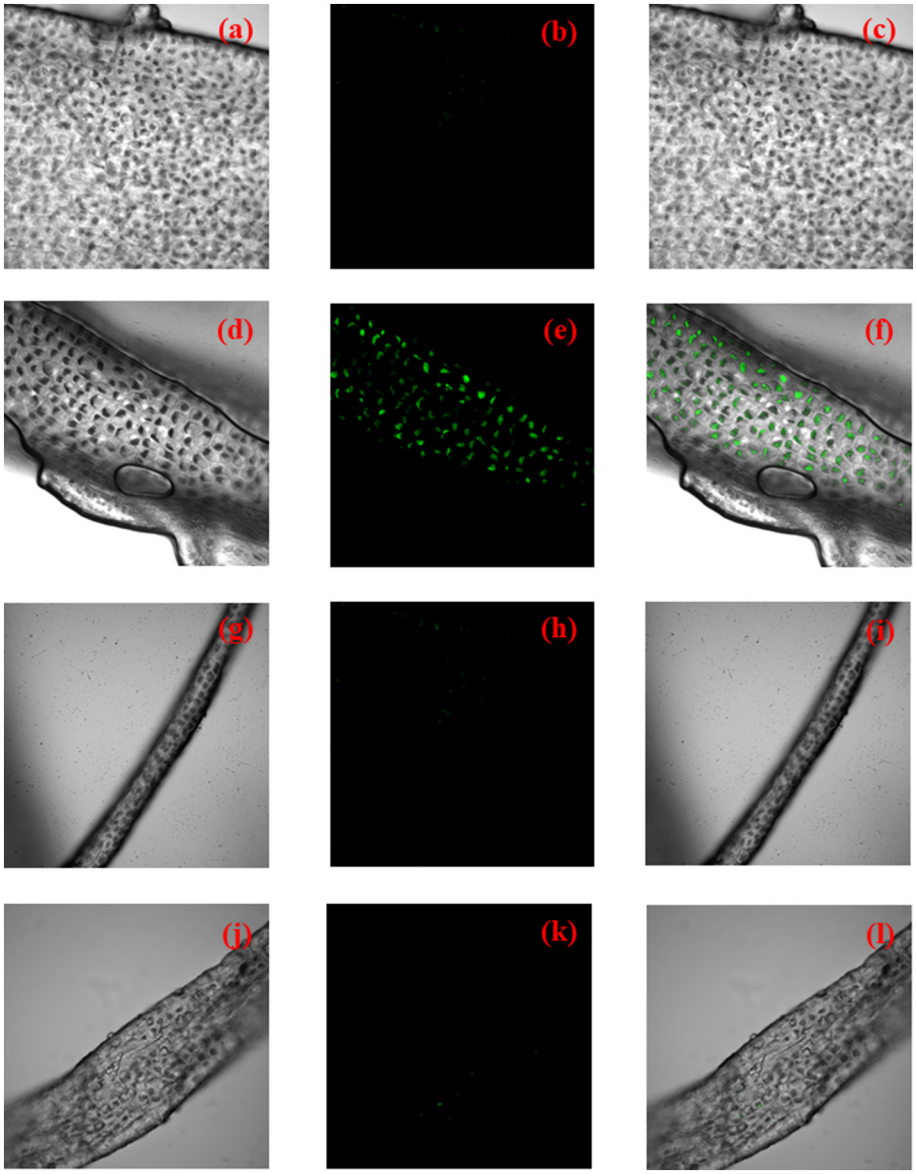

Fig. 2. Laser confocal scanning microscopy (LCSM) image of the control without bisphenol A in both live (a, b, c) and heat-killed (g, h, i) *U. prolifera*, and bisphenol A distributed in both live (d, e, f) and heat-killed (j, k, l) *U. prolifera* under brightfield image (a, d, g, j), green fluorescence image (b, e, h, k) and overlay image (c, f, i, l) (×40, $\lambda_{ex06} = 405$ nm). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

comparisons showed that BPA removal efficiency by *U. prolifera* had no significantly differences ($p > 0.05$) among salinity variations. As an intertidal species, *U. prolifera* possessed a wide salinity tolerance (Rybak, 2018). Therefore, salinity variation had no effect on the removal of BPA.

3.3. Effect of nutrient and temperature on BPA removal

According to our previous survey for coastal waters along nearly 18,000 km of coastline of China, concentrations of NO_3^--N and PO_4^{2-}-P were in the ranges of 1.58–107.61 μM and 0.18–5.07 μM, respectively. In areas adjacent to aquaculture, the concentration of PO_4^{2-}-P reached 36.14 μM. Therefore, the removal of BPA by *U. prolifera* under three different nutrient concentrations were evaluated (Fig. 4). After 24 h exposure, the removal efficiencies at low ((0 μM (NO_3^-)/0 μM (PO_4^{2-})), middle ((50 μM (NO_3^-)/5 μM (PO_4^{2-})), and high ((150 μM (NO_3^-)/15 μM (PO_4^{2-})) nutrient levels were 94.3%, 97.3%, and 99.9%, respectively. Results from One-way ANOVA analysis showed that nitrogen/phosphate enrichment could significantly facilitate ($p < 0.05$) the BPA removal rate at three nutrient concentrations. The BPA removal rate (k values changed from 0.123 to 0.234 h^{-1}) increased as the nutrient concentration increased (Fig. 4 inset) and reached the highest value at the highest nutrient level (150 μM (NO_3^-)/15 μM (PO_4^{2-})). It was safe to argue the high potential in BPA removal during the eutrophic period of the coastal waters since algae species, *U. prolifera*, survived bloomy in the water environment with high nutrient concentration.

The influence of temperature on the removal of BPA by *U. prolifera* was also investigated (Fig. 5). Specifically, one-way ANOVA results showed that there was a rapid increase trend as the temperature changed from 10 to 20 °C and then a slightly increase trend as the temperature rose to 30 °C. The maximum removal constant k (0.129 h^{-1}) was acquired under the highest temperature (30 °C) treatment, which was almost 1.5 times larger than the treatment under 10 °C (k = 0.088 h^{-1}) (Fig. 5 inset). The increase in temperature was beneficial to the removal of BPA, which was similar with other report on the

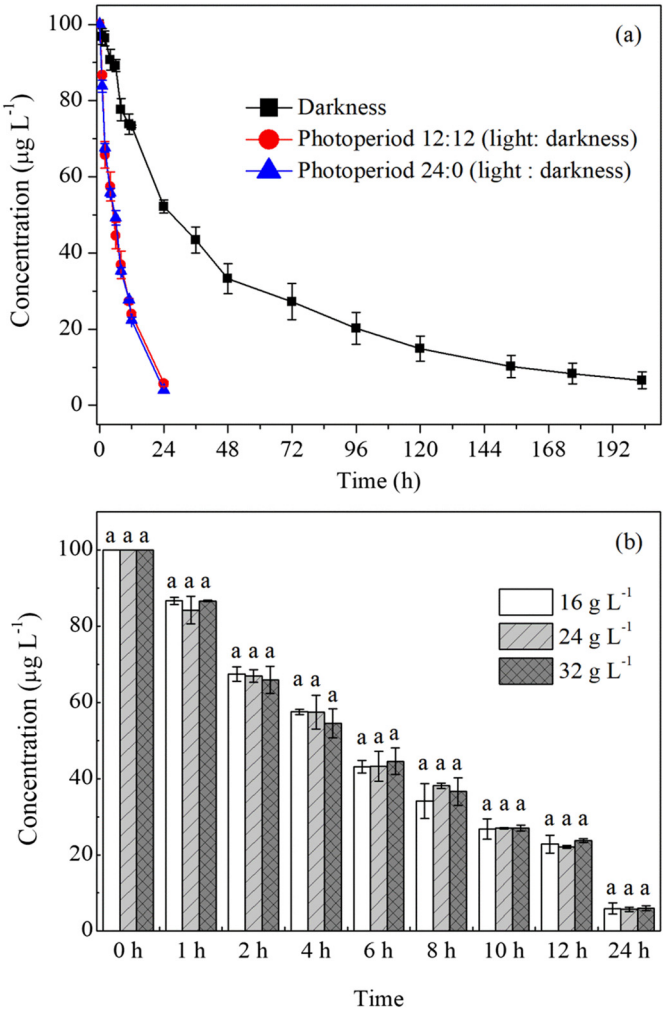

Fig. 3. Removal of bisphenol A by *U. prolifera* at different (a) photoperiods and (b) salinity. Same letters on top of the bar at the same sampling time means that do not significantly differ ($p > 0.05$) from each other based on one-way ANOVA test.

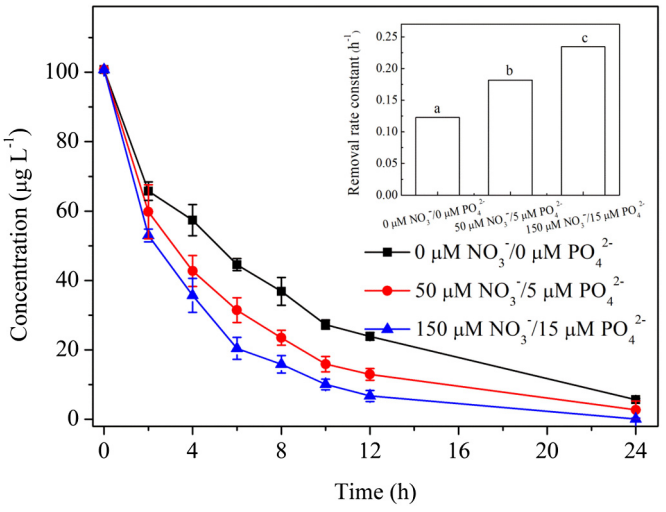

Fig. 4. Removal of bisphenol A at different nutrient concentration by *U. prolifera*. Inset: First-order rate constant (k) for uptake removal at different nutrient concentration. Bars shared the different letter indicate the means are notably different among the salinity at $p < 0.05$, while same letters on top of the bar at the same sampling time means that do not significantly differ ($p > 0.05$) from each other according to one-way ANOVA test.

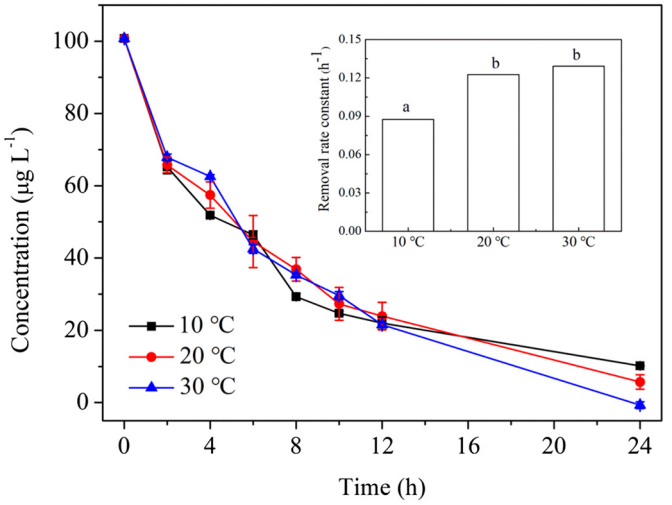

Fig. 5. Effect of temperature on the removal of bisphenol A by *U. prolifera*. Inset: First-order rate constant (k) at different temperatures. Bars shared the different letter indicate the means are notably different among the salinity at $p < 0.05$, while same letters on top of the bar at the same sampling time means that do not significantly differ ($p > 0.05$) from each other according to one-way ANOVA test.

remazol black B dye removal by green alga *Chlorella vulgaris* (Aksu and Tezer, 2005).

3.4. Effect of initial concentration of BPA on BPA removal

The removal of BPA in the presence of *U. prolifera* was evaluated under treatments using different initial BPA concentrations. The BPA was removed efficiently in algal system as the time proceeded (Fig. 6). The removal efficiency of BPA by *U. prolifera* increased from 94.30% to 99.81% as initial BPA concentration increased from 50 to 1000 μg L^{-1} during 5 days. It was acknowledged that the resistances of the BPA between two phases (aqueous and algal phases) could be overcome by the driving force obtained from a higher initial substance (Aravindhan et al., 2007) to subsequently increase the uptake. In addition, average removal rate was fitted as a function of initial concentration in this study. Increased average removal rate was observed with increase of initial BPA concentration (Fig. 6 inset). A linear relationship was also accomplished between them ($R^2 = 0.999$). The maximum average

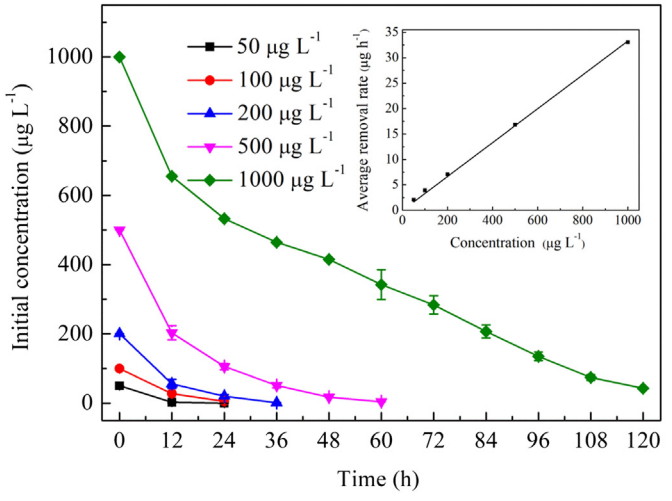

Fig. 6. Removal of bisphenol A by *U. prolifera* at different initial bisphenol A concentrations. Inset: Average removal rate as a function of the initial concentration of bisphenol A ($R^2 = 0.999$).

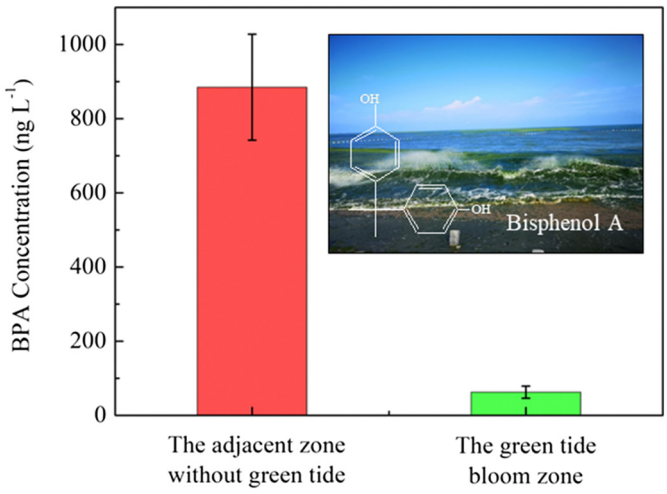

Fig. 7. Field investigation on concentrations of bisphenol A in the coastal waters of the green tide blooming zone and the adjacent zone without green tide. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

removal rate reached 33.04 μg h^{-1} when the initial BPA concentration was 1000 μg L^{-1} within 24 h exposure. It is generally accepted that the removal rate increases as linearity to the initial concentration of contaminants only at very low initial concentrations while high substrate concentration might reduce the removal efficiency by organisms because of their possible toxicity (Lu et al., 2008). However, the linear relationship maintained even at relatively high initial concentration, indicating that *U. prolifera* had a high tolerance to the toxic effect of BPA pollution.

According to previous investigations, concentrations of BPA detected in various mediums range from ng L^{-1} in sewage effluents (Xu et al., 2014) and surface water (Yamazaki et al., 2015) to mg L^{-1} in several landfill leachates (Yamamoto et al., 2001). Since high BPA removal efficiency (>94%) was achieved at environmental relevant concentrations, *U. prolifera* might have the potential of efficiently removing BPA from coastal water within its floating area during the outbreak of green tide. Due to the high biomass (8.47 g m^{-2}) and huge covered area (3.6×10^4 km^2) of the *U. prolifera* during the outbreak of green tide (Liu et al., 2015), the contribution of the green-tidal algae in the removal of BPA in the coastal and marine waters should be considerable.

3.5. Field investigation

To obtain further evidence on the phycoremediation of coastal waters containing BPA, the regional differences in the concentrations of BPA in the coastal waters were evaluated between the green tide blooming zone and the adjacent zone without green tide (control investigation). The concentration of BPA was detected as 62.38 ± 16.33 ng L^{-1} in the green tide blooming zone, much lower than that (884.86 ± 142.82 ng L^{-1}) in the adjacent zone without green tide (Fig. 7). These results confirm that phycoremediation by *U. prolifera* is an important new natural remediation process for coastal waters contaminated with BPA during green tide blooming.

4. Conclusion

A new natural phycoremediation process for BPA in coastal waters during green tide bloom was illustrated. BPA could be efficiently removed in the presence of green-tidal algae, *U. prolifera*. The environmental factors including light, nutrient and temperature were positively correlated with BPA removal efficiency. However, salinity variation scarcely influenced the removal of BPA. A linear relationship was observed between average removal rate and BPA initial concentration in this study. Additionally, high removal efficiency (>94%) was obtained under environmentally relevant concentrations of BPA to suggest that most of BPA might be removed from coastal water with such high biomass and huge covered area of *U. prolifera* during green tide blooming. Compared with the adjacent zone without green tide, much lower BPA concentrations in the coastal waters of the green tide blooming zone were detected. These findings confirm that phycoremediation by *U. prolifera* is a new potential phycoremediation process for coastal waters contaminated with BPA during the green tide blooming.

Acknowledgements

This work was supported by National Natural Science Foundation of China (grant numbers of 41671319 and 41877131), One Hundred Talents Program of Chinese Academy of Sciences (grant numbers of Y629041021 and Y610061033), Taishan Scholar Program of Shandong Province, and Two-Hundred Talents Plan of Yantai (Y739011021).

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