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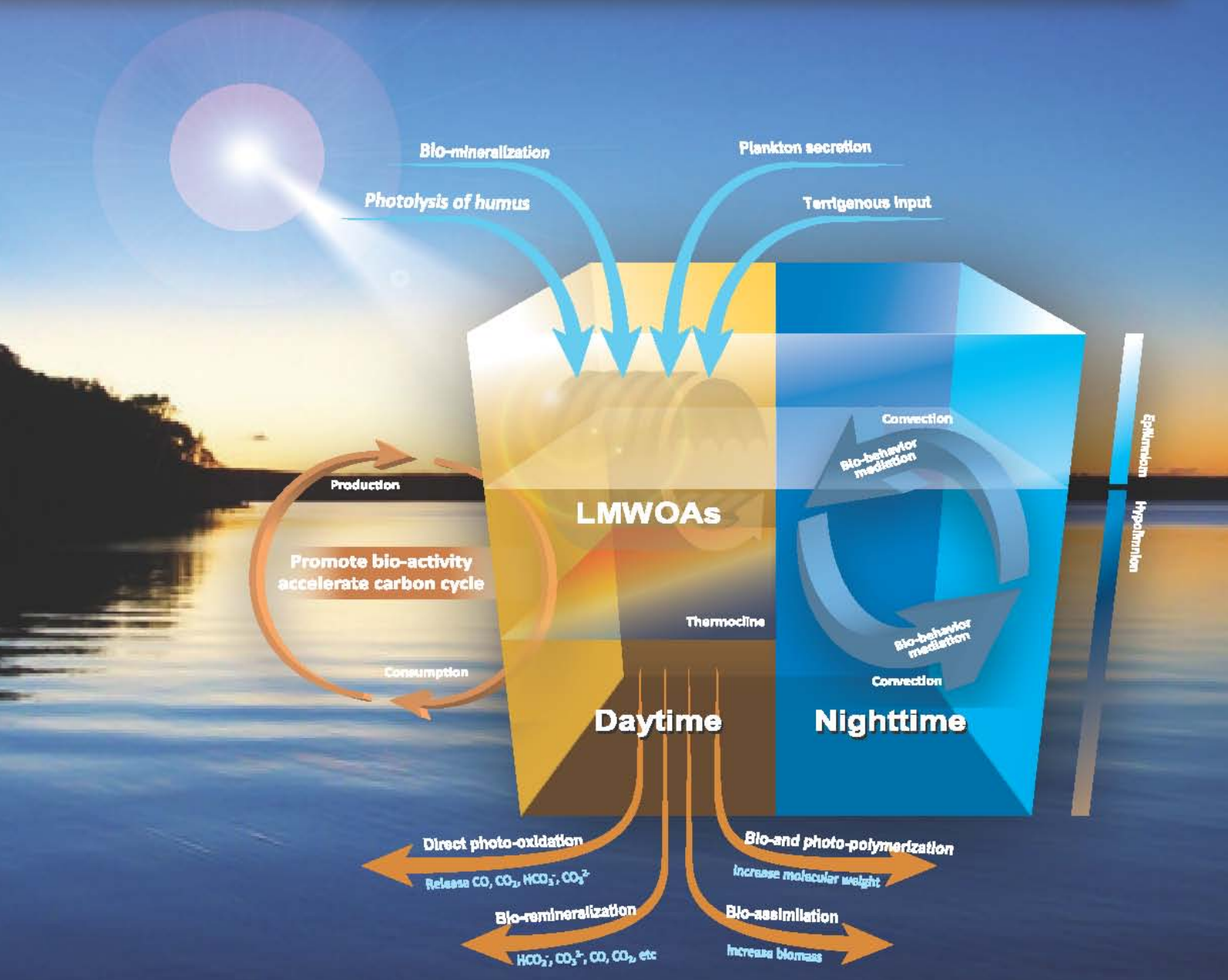

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CONTENTS

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Effects of nitrogen and phosphorus concentrations on the bioaccumulation of polybrominated diphenyl ethers by *Prorocentrum donghaiense*

Chao Chai[1,*], Xundong Yin[1], Wei Ge[2], Jinye Wang[3]

1. College of Resources and Environment, Qingdao Agricultural University, Qingdao 266109, China. E-mail: chaichao1999@126.com
2. College of Life Sciences, Qingdao Agricultural University, Qingdao 266109, China
3. College of Animal Science and Veterinary, Qingdao Agricultural University, Qingdao 266109, China

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Abstract

The growth, cellular total lipids, bioaccumulation amount, and bioaccumulation factors (BAFs) of 2,4,4′-tribromodiphenyl ether (BDE28), 2,2′,4,4′-tetrabromodiphenyl ether (BDE47), and 2,2′,4,4′,5-pentabromodiphenyl ether (BDE99) in a semi-continuous culture of *Prorocentrum donghaiense* were studied in relation to nitrate (0, 128, and 512 μmol/L) and phosphate (0, 8, and 32 μmol/L) concentrations. The BDE28, BDE47, and BDE99 content per cell under 0 μmol N/L were 3.77×10^{-6}, 3.95×10^{-6}, and 4.32×10^{-6} ng/cell, respectively, which were significantly higher than those under 128 and 512 μmol N/L. A nearly 5-fold increase in polybrominated diphenyl ether (PBDE) content per algal cell was found between 0 and 8 μmol P/L and between 8 and 32 μmol P/L. With increasing N and P concentrations, the PBDE content per volume of algal culture and the accumulation percentage of available PBDEs declined slightly. The BAFs for the PBDEs based on lipids showed that the $\log BAF_{lip}$ under 0 μmol N/L was higher than those under 128 and 512 μmol N/L. The $\log BAF_{lip}$ under 0 μmol P/L was higher than that under 8 μmol P/L but lower than that under 32 μmol P/L. Correlation analysis indicated a significant negative correlation between nutrient concentration and cellular total lipids, as well as the PBDE content per cell. The results indicate that different N and P concentrations change the total lipids content of *P. donghaiense*, thereby resulting in varying PBDE accumulation.

Key words: nitrogen; phosphorus; bioaccumulation; polybrominated diphenyl ethers; *Prorocentrum donghaiense*

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Introduction

Polybrominated diphenyl ethers (PBDEs) are widely used as brominated flame retardants in consumer products such as electronics, electrical equipment, and textiles since the 1970s (Rahman et al., 2001). The properties of PBDEs include lipophilicity, persistence, bioaccumulative potential, and endocrine disruption in humans and wildlife (Gustafsson et al., 1999; Darnerud et al., 2001; McDonald, 2002; Richardson et al., 2008). In 1979, PBDEs were first detected in the soil near a PBDE manufacturing plant in the US (DeCarlo, 1979). Thus far, PBDEs have been found in the atmosphere, fresh and marine water, sediment, animal fat, human blood, and breast milk (Sellström et al., 1993; Hale et al., 2003; Ohta et al., 2002; Yang et al., 2004; Suzuki et al., 2006; Law et al., 2006; Borghesi et al., 2008; Luo et al., 2008; Jin et al., 2008; Guan et al., 2011). The PBDE levels in these samples have increased rapidly in the past 30 years (Luross et al., 2000; Moisey et al., 2001; Stern and Ikonomou, 2000; Ikonomou et al., 2002; She et al., 2002). Therefore, PBDEs have become a globally notorious contaminant and concerns over the adverse effects of PBDE exposure continue to increase.

The PBDE family consists of 209 congeners. Lower brominated PBDEs (less than 5 bromine atoms per molecule) accumulate at a higher content in aquatic life than higher brominated PBDEs because they are more efficiently bioaccumulated or have higher biological availability (De Wit, 2002; De Wit et al., 2006; Wang et al., 2007). Therefore, we used three lower brominated PBDEs congeners, 2,4,4′-tribromodiphenyl ether (BDE28), 2,2′,4,4′-tetrabromodiphenyl ether (BDE47), and 2,2′,4,4′,5-pentabromodiphenyl ether (BDE99), which accumulate at relatively high levels in aquatic life, to observe the bioaccumulation of PBDEs by phytoplankton.

Phytoplankton, the primary producers in aquatic ecosystems, plays an important role in the transport and fate of persistent organic pollutants (POPs). Phytoplankton drives POP transfer by bioaccumulation, followed by biomag-

* Corresponding author. E-mail: chaichao1999@126.com

nification, because they are rich in lipids and are at the bottom of the aquatic food chain. The extent of POP bioaccumulation by phytoplankton is related to the characteristics of organic substances, phytoplankton species, biomass, lipid content, and environmental factors (Stange and Swackhamer, 1994; Carlson and Swackhamer, 2006; Berglund et al., 2000). Berglund et al. (2001) found a close relationship between the levels of polychlorinated biphenyls (PCBs) amount and the lipid content in microalgae in 19 lakes in Southern Sweden. Lipid content was influenced by lake trophism. Many studies have reported changes in the lipid content of microalgal cells under different nutrient statuses (Hu et al., 2008; Merzlyak et al., 2007; Zhao et al., 2009; Li et al., 2010). Halling-Sørensen et al. (2000) found that nitrogen deficiency increases algal lipids, which influences PCB bioaccumulation by the freshwater green algae *Selenastrum capricornutum*. Lynn et al. (2007) found the PCB bioaccumulation factor (BAF) of the freshwater diatom *Stephanodiscus minutulus* under silica-limited conditions is higher than under non-limited silica conditions. These studies mainly focused on PCB bioaccumulation by freshwater phytoplankton in nutrient-deficient settings. However, few studies have examined the nutrient concentration–induced changes in PBDE bioaccumulation in marine dinoflagellates.

The dinoflagellate *Prorocentrum donghaiense* is a common and dominant harmful algal bloom species. It has caused large-scale red tides along coastal areas in China in recent years, with the increasing input of nitrogen and phosphorus (Tang et al., 2006). The objective of this study is to determine whether the bioaccumulation of BDE28, BDE47, and BDE99 by *P. donghaiense* changes with the algal total lipids levels under different nitrogen and phosphorus concentrations.

1 Materials and methods

1.1 Algal culture and growth conditions

Prorocentrum donghaiense was isolated from the adjacent East China Sea. The nonaxenic cell population was grown in sterilized seawater enriched with F/2 medium. The stock culture was maintained in the exponential growth phase by routine subculture. The stock and experimental cultures were maintained at (20 ± 1)°C on a 12 hr:12 hr light:dark cycle at 45 μmol/(m^2·sec) supplied by cool-white fluorescent tubes.

1.2 Experimental design

The glassware were washed with 30% HCl (*V*/*V*) to remove possible contaminants, rinsed with distilled water, and then autoclaved (121 kPa, 30 min). Cultures in the exponential growth phase were inoculated into 3 L of sterilized artificial seawater in 5 L flasks with different nitrogen (N) and phosphorus (P) concentrations. Before the inoculation, the algal cells were grown in sterilized seawater without N and P for 48 hr to ensure that the N and P in the medium were exhausted. The response to different N concentrations was determined using 0, 128, and 512 μmol/L $NaNO_3$. The P concentration was 36 μmol/L NaH_2PO_4 in all N treatments. The response to different P concentrations was determined using 0, 8, and 32 μmol/L NaH_2PO_4. The N concentration was maintained at 883 μmol/L $NaNO_3$ in all P treatments. Other nutrients were added according to the requirements of the F/2 medium in all N and P treatments. The cultures were diluted with sterilized artificial seawater containing different N and P concentrations once a day via the semicontinuous approach (600 mL/day), giving a 0.2/day exchange rate. Each treatment was conducted in triplicate.

The BDE28, BDE47, BDE99 stock solutions (50 μg/mL) were purchased from AccuStandard, Inc. (USA). The stock solutions were diluted with methanol, resulting in 0.2 μg/mL PBDE working solution. After the media exchange on day 6 of algal cultivation, the 3 L cultures were divided into three of 1 L cultures. Three working PBDE solutions were added to 1 L cultures. The PBDE concentration in the algal cultures was 0.2 μg/L and the exposure time was 24 hr.

1.3 Sampling and analytical methods

Before the daily media exchange, 20 mL subsamples were obtained and fixed with Lugol's solution for cell counts. Then, 100 mL subsamples were filtered through a 0.45 μmol/L micropore filter membrane to determine the both nitrate and phosphate concentrations. After 24 hr of exposure to PBDEs, the 200 mL subsamples were centrifuged for 20 min at 8000 r/min. The harvested cells were rinsed with ammonium formate and immediately stored at –20°C for total lipids content analysis. The subsamples (250 mL) were filtered through fiberglass Whatman GF/F filters (precombusted at 450°C for 7 hr), which were frozen for PBDE extraction and analysis.

The filtrates for the N and P determination were analyzed after sampling each day. Inorganic nitrate was measured via the cadmium-copper reduction method (Grasshoff, 1976), whereas phosphate was measured using phosphomolybdenum blue (Strickland and Parsons, 1972).

The total lipids content was extracted according to Bligh and Dyer (1959) and it was quantified using the method described by Pande et al. (1963). The calibration curve was constructed using palmitic acid as the standard for total lipids.

The samples for PBDE determination were Soxhlet-extracted with hexane for 48 hr. Before extraction, the samples were spiked with 50 ng of 2,2′,4,4′,5,5′-hexabromodiphenyl ether (BDE153) as the surrogate standard, whereas activated copper was added to the flask for desulfurization. The extracts were concentrated to 2 mL and were cleaned according to the method by Luo et al. (2008). A multilayer silica/alumina column

with a 10 mm i.d. glass column containing activated alumina, neutral silica gel, alkaline silica gel, acidic silica gel, and anhydrous sodium sulfate was used for purification. The eluent hexane:dichloromethane (1:1, *V*/*V*) was concentrated, solvent-exchanged to isooctane, and further reduced to 0.1 mL under a gentle stream of N_2. Prior to instrumental analysis, 10 μL (1 μg/mL) of 2,2′,4,5′,6-pentachlorobiphenyl (PCB103) was added to all extracts as the internal standard.

Instrumental analysis was carried out on an Agilent 6890N gas chromatograph (GC) equipped with electron capture detector. The GC column used was an HP-5 column (30 m × 0.25 mm i.d., 0.25 μm film thickness). The temperature of the oven was first held at 150°C for 1 min, then heated at 40°C/min to 250°C, and heated at 10°C/min to 300°C for 7 min. The injector temperature was set to 300°C, and the GC was operated in splitless injection mode (Fontana et al., 2009).

The PBDE concentrations were calculated using multiple-peak linear regression analysis. Six standard samples for each congener were used for the calibration curves. A standard was analyzed every 10 samples to determine instrument stability and to confirm the calibration curve.

The solvents used in PBDE analysis were chromatographically pure. Quality assurance was done by analyzing procedural blanks every 10 samples and surrogate standards for each sample. The target PBDE levels in procedural blanks corresponded closely to the limit of quantification; thus, they were not subtracted from those in the samples. The mean recoveries of the surrogate standards were 82% ± 9%. No surrogate corrections were made to the final concentrations reported.

1.4 Statistical analysis

The specific growth rate (μ, day^{-1}) was estimated according to Eq. (1) (Landry and Hassett, 1982):

$$\mu = \frac{\ln(N_n/N_0)}{t_n - t_0} \quad (1)$$

where, N_n and N_0 are the cell densities at the time t_n and t_0, respectively.

Bioaccumulation factors (BAFs) were based on the total lipids content (BAF_{lip}, Lynn et al., 2007) and calculated as follows:

$$BAF_{lip} = \frac{C_{PBDFs\text{-}c}/C_{lipids\text{-}c}}{C_{PBDEs\text{-}m}} \quad (2)$$

where, $C_{PBDEs\text{-}c}$ (ng/mL) and $C_{lipids\text{-}c}$ (g/mL) are the contents of PBDEs and lipids in culture, respectively, the $C_{PBDEs\text{-}m}$ (ng/mL) is the PBDEs content in the media and was calculated by subtracting the PBDE mass in the algal cells from the nominal whole flask PBDE mass.

The mean differences were analyzed by ANOVA, and significantly different means were separated (P = 0.05) using the least significant difference test. Correlation analysis was carried out using Pearson's correlation at a significance level of α = 0.05. Statistical analyses were performed using the SPSS 16.0 software.

2 Results

2.1 Algal growth

The cell density under all N concentrations presented similar trends during the early culture period (**Fig. 1**). On day 7, the cell densities under the 128 and 512 μmol N/L treatments were about 3–4 times as high as that under the 0 μmol N/L treatment. However, no significant difference was observed between the 128 and 512 μmol N/L treatments. Similarly, the cell densities on day 7 under the 8 and 32 μmol P/L treatments were about 5 times as high as that under the 0 μmol P/L treatment.

On the last day of cultivation, the specific growth rate was nearly 0 day^{-1} under the 0 μmol N and P/L treatments (**Fig. 1c, 1d**). However, it was about 0.3 day^{-1} under other N and P concentrations.

2.2 Nutrient concentrations

After 7 days, the nitrate concentrations decreased from 512 to 394 μmol/L and from 128 to 4.5 μmol/L. No significant difference was observed under the 0 μmol N/L treatment (**Fig. 2a).** The phosphate concentration in the media in all N treatments ranged from 24 to 36 μmol/L. In the P treatments, the phosphate concentration decreased from 32 to 19.1 μmol/L and from 8 to 0.1 μmol/L, but remained unchanged in the 0 μmol P/L treatment (**Fig. 2b**). The nitrate concentrations in all P treatments were higher than 800 μmol/L in the media.

Justin et al. (1995) reported that the threshold for dissolved inorganic nitrogen of phytoplankton growth was 1 and 0.1 μmol/L for dissolved phosphate. Only the culture under the 0 μmol N/L concentration was severely N-deficient, whereas all cultures in the N treatments were not limited by P. The algal cultures under the 0 and 8 μmol P/L concentrations had severe and potential P deficiencies, respectively, and no N deficiency was observed in all P treatments.

2.3 Total lipids content

The cellular total lipids content declined with increasing N and P concentrations (**Fig. 3**) and was nearly 0.1 × 10^{-6} mg/cell under 0 μmol N/L, which is significantly higher than those under 128 μmol N/L (0.04 × 10^{-6} mg/cell) and 512 μmol N/L (0.03 × 10^{-6} mg/cell) (P < 0.05). A 2.5-fold to 3.5-fold increase was observed from higher N to 0 μmol N/L concentrations (**Fig. 3**). The total lipids content under the three P treatments exhibited trends similar to those under the N treatments, but the differences were more significant. The total lipids content were 0.23 × 10^{-6}, 0.06 × 10^{-6}, and 0.03 × 10^{-6} mg/cell under 0, 8, and

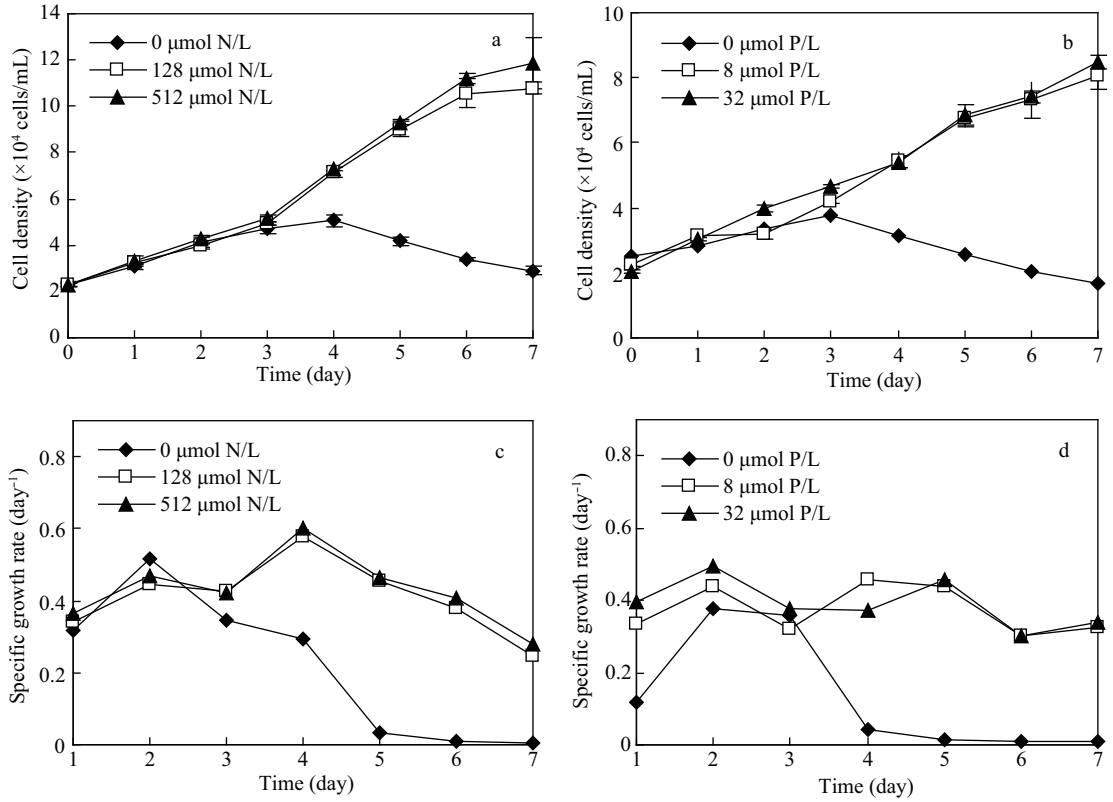

Fig. 1 Cell density (a, b) and specific growth rate (c, d) of *P. donghaiense* under different N and P concentrations.

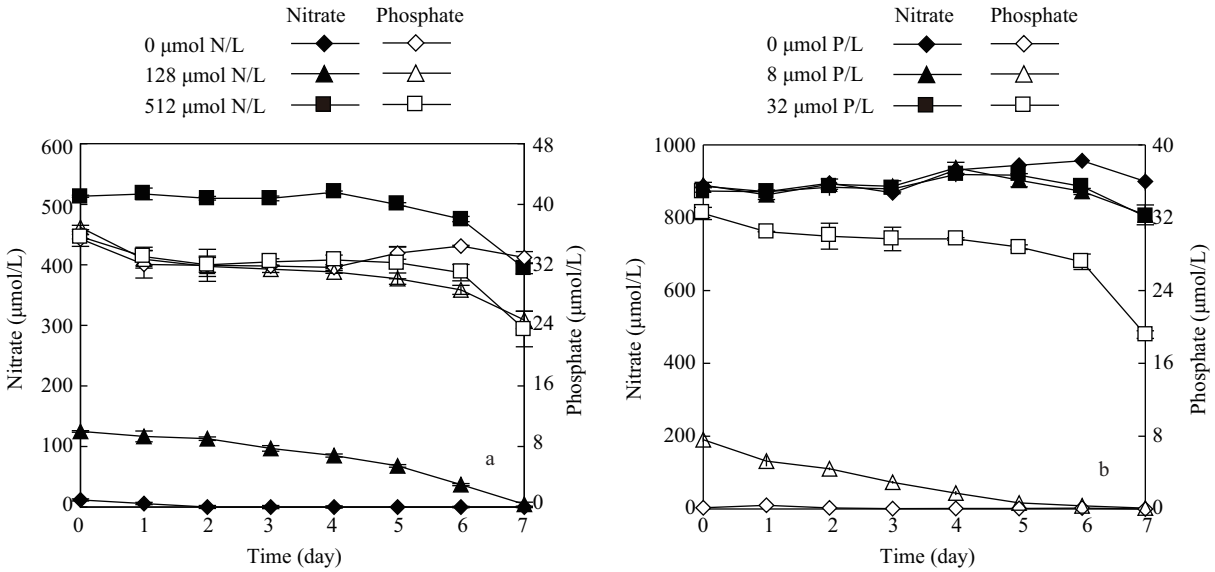

Fig. 2 Changes in N and P concentrations in the *P. donghaiense* culture under different N (a) and P (b) treatments.

32 μmol P/L, respectively. The 4-fold and 8-fold increases were observed between 8 and 0 μmol P/L and between 32 and 0 μmol P/L, respectively (**Fig. 3b**).

2.4 PBDE bioaccumulation

The PBDE levels per cell under 0 μmol N/L and 0 μmol P/L were significantly higher than those under other N and P concentrations (**Fig. 4a**). At an N concentration of 0 μmol/L, BDE28, BDE47, and BDE99 content were 3.77×10^{-6}, 3.95×10^{-6}, 4.32×10^{-6} ng/cell, respectively, which were significantly higher than those under 128 and 512 μmol N/L ($P < 0.05$). The PBDE content per cell under different P concentrations exhibited trends similar to those under the N treatments. A nearly 5-fold increase in PBDE content was found between 8 and 0 μmol P/L, and between 32 and 0 μmol P/L.

The difference in PBDE content per volume of algal culture was not as significant as the PBDE content per

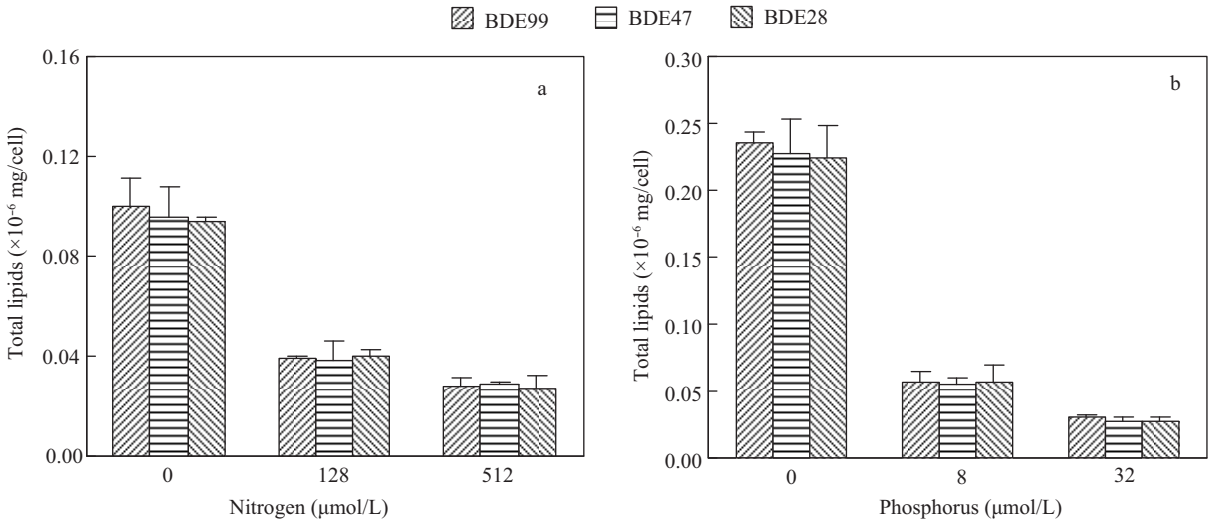

Fig. 3 Total lipids content of *P. donghaiense* under different N (a) and P (b) concentrations.

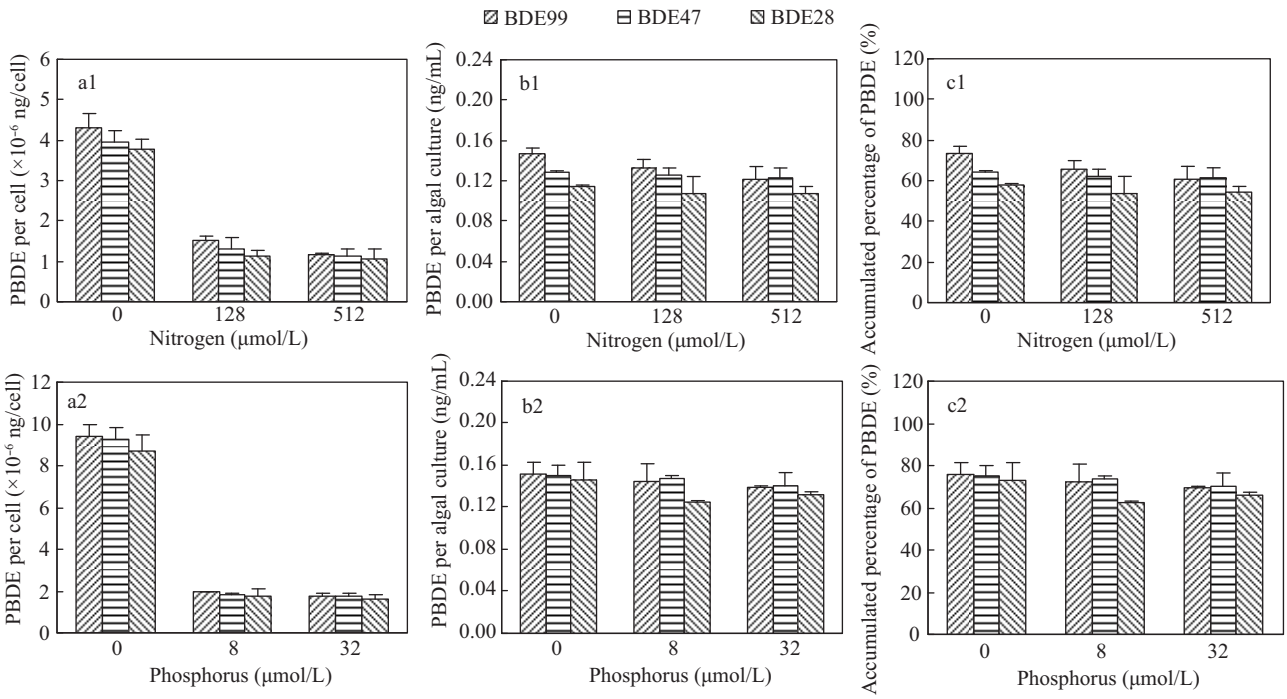

Fig. 4 PBDE content per cell (a1, a2) and per volume (b1, b2) of *P. donghaiense* and accumulation percentage of available PBDEs (c1, c2) by *P. donghaiense* under different N and P concentrations.

cell under the different N and P concentrations (**Fig. 4b**). With increasing N and P concentrations, the PBDE content exhibited a slightly declining trend. The PBDE content per culture were 0.115 to 0.147 ng/mL, 0.107 to 0.132 ng/mL and 0.108 to 0.123 ng/mL under 0, 128, and 512 μmol N/L, respectively. Furthermore, the PBDE levels per culture were 0.146 to 0.152 ng/mL, 0.125 to 0.147 ng/mL, and 0.132 to 0.140 ng/mL under 0, 8, and 32 μmol P/L, respectively.

The accumulation percentage of available PBDEs exhibited a trend similar to that of the PBDE per volume of algal culture (**Fig. 4c**). With increasing N and P concentrations, accumulation percentage of *P. donghaiense* declined. However, the differences among the different N or P concentrations were not significant ($P > 0.05$).

Table 1 presents the BAFs based on the lipids content (BAF_{lip} and $logBAF_{lip}$) under all nutrient concentrations. The $logBAF_{lip}$ under 0 μmol N/L was higher than those under 128 and 512 μmol N/L. The $logBAF_{lip}$ under 0 μmol P/L was higher than that under 8 μmol P/L but lower than that under 32 μmol P/L.

2.5 Relationship among nutrient concentration, cellular total lipids, and bioaccumulation amount

The Pearson's correlation analysis indicated that the N and P concentrations were negatively correlated with the

Table 1 BAFs of PBDEs by *P. donghaiense* under different N and P concentrations

Nutrient	Concentration (μmol/L)	BAF_{lip} ($\times10^4$ ng/g)/(ng/mL)			$logBAF_{lip}$		
		BDE99	BDE47	BDE28	BDE99	BDE47	BDE28
N	0	89.7 ± 9.4 a*	59.2 ± 3.3 ad	46.6 ± 8.0 ae	5.95 ± 0.05 a	5.77 ± 0.02 ad	5.67 ± 0.07 ae
	128	44.3 ± 8.4 b	42.7 ± 9.7 b	25.8 ± 2.4 b	5.65 ± 0.08 b	5.63 ± 0.10 b	5.41 ± 0.04 b
	512	50.9 ± 5.5 b	47.3 ± 4.3 bd	40.0 ± 6.8 a	5.71 ± 0.08 b	5.68 ± 0.04 bd	5.60 ± 0.08 a
P	0	71.1 ± 7.6 c	67.8 ± 11.7 d	57.5 ± 4.2 de	5.85 ± 0.05 c	5.83 ± 0.08 d	5.76 ± 0.03 de
	8	54.5 ± 9.3 b	60.3 ± 3.8 d	35.8 ± 10.1 ab	5.74 ± 0.08 b	5.78 ± 0.03 d	5.55 ± 0.13 ab
	32	93.1 ± 10.5 a	100.0 ± 3.7 c	85.3 ± 4.7 c	5.97 ± 0.05 a	6.00 ± 0.02 c	5.93 ± 0.02 c

* Letters indicate statistical differences between nutrient conditions. Values that do not share a common letter are significantly different ($P < 0.05$).

cellular total lipids (N: $r = –0.791$, $P < 0.05$; P: $r = –0.781$, $P < 0.05$) and PBDE content per cell (N: $r = –0.729$, $P < 0.05$; P: $r = –0.704$, $P < 0.05$). On the other hand, the cellular total lipids was positively correlated with the PBDE content per cell (N: $r = 0.991$, $P < 0.01$; P: $r = 0.993$, $P < 0.01$; **Table 2**).

3 Discussion

3.1 Accumulated amount of PBDEs under different N and P concentrations

Datta et al. (2001) and Lynn et al. (2007) found that the uptake of 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB101) by *Cyclotella meneghiniana* and 2,2′,6,6′-tetrachlorobiphenyl (PCB54) by *S. minutulus* under Si-, N-, and P-limited conditions was higher than those under non-limited conditions. Kilham (1998) reported that under Si limitation, the diatom *Nitzschia* sp. accumulated about twice the amount of tritiated polychlorinated dibenzofurans (PCDFs) as that under non-limited conditions, whereas the accumulated PCDFs under P limitation was less than those under non-limited conditions. In the present study, *P. donghaiense* accumulated about 3-fold and 5-fold the amount of PBDEs under 0 μmol N and P/L, respectively, compared with those under higher N and P concentrations (**Fig. 4a**). This result may be attributed to the total lipids levels, which was significantly higher under the initial 0 μmol N and P/L concentrations than those under other N and P concentrations (**Fig. 3**). The total lipids content of the phytoplankton increased under nutrient-deficient conditions (Hu et al., 2008; Merzlyak et al., 2007; Zhao et al., 2009; Li et al., 2010), whereas the bioaccumulation of hydrophobic organic chemicals was related to the lipids content of the phytoplankton and other organisms (Geyer et al., 1984; Manthey et al., 1993; Meador et al., 1995; Magnusson et al., 2007). Correlation analysis indicated that nutrient concentration was negatively correlated with the cellular total lipids and the PBDE amount. In addition, cellular total lipids were significantly positive correlated with accumulated PBDE levels (**Table 2**). The results of this study indicate that different N and P concentrations induced changes in total lipids content of *P. donghaiense*, which resulted in variations in PBDE accumulation.

3.2 PBDE BAFs under different N and P concentrations

BAF is the ratio of the compound concentration in the organism to that in the water. At equilibrium, BAF is called bioconcentration factors (BCF). BAFs or BCFs for hydrophobic organic compounds in phytoplankton are affected by nutrient status. The lipid-normalized BCFs for the four PCB congeners of the green algae *S. carpricornutum* increased 1.5–8.9 times under nitrogen starvation (Halling-Sørensen et al., 2000). Lynn et al. (2007) found that the lipid-normalized BAF for PCB54 of *S. minutulus* under Si limitation was higher than that under non-limited conditions. However, those under limited N and P were lower. In this study, the BAF_{lip} of *P. donghaiense* for the three PBDEs under 0 μmol N/L increased by 1.2-fold to 2-fold. This result is similar to BCFs of

Table 2 Pearson correlation analysis among N, P concentrations, total lipids, and bioaccumulation amount of *P. donghaiense* ($n = 27$)

	N	$Lipid_T$	$PBDE_{ce}$	$PBDE_v$	$PBDE_a$	P	$Lipid_T$	$PBDE_{ce}$	$PBDE_v$	$PBDE_a$
N/P	1					1				
Sig. (2-tailed)										
$Lipid_T$	–0.791*	1				–0.781*	1			
Sig. (2-tailed)	0.011					0.013				
$PBDE_{ce}$	–0.729*	0.991**	1			–0.704*	0.993**	1		
Sig. (2-tailed)	0.026	0.000				0.034	0.000			
$PBDE_v$	–0.393	0.476	0.541	1		–0.492	0.639	0.651	1	
Sig. (2-tailed)	0.295	0.195	0.133			0.179	0.064	0.058		
$PBDE_a$	–0.396	0.484	0.548	1.000**	1	–0.492	0.639	0.651	1.000**	1
Sig. (2-tailed)	0.291	0.187	0.126	0.000		0.179	0.064	0.058	0.000	

$Lipid_T$: cellular total lipids content; $PBDE_{ce}$: PBDE content per cell; $PBDE_v$: PBDE per volume of culture; $PBDE_a$: accumulated percentage of available PBDE.

* Correlation is significant at the 0.05 level (2-tailed); ** correlation is significant at the 0.01 level (2-tailed).

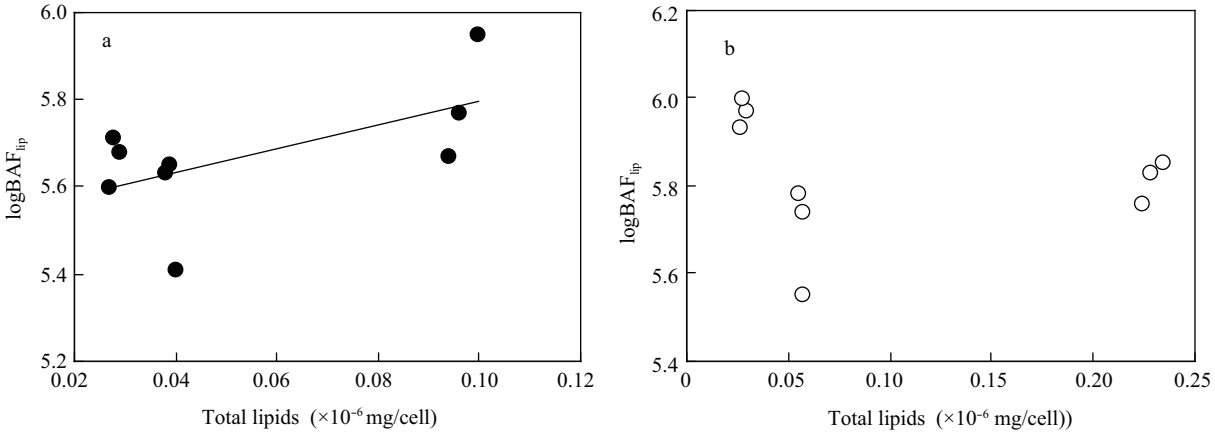

Fig. 5 Relationship between the BAF_{lip} and the cellular total lipids content of *P. donghaiense* under different N (a) and P (b) concentrations.

S. carpricornutum for 2,4′,5-trichlorobiphenyl (PCB31), 2,2′,4,5′-tetrachlorobiphenyl (PCB49), and 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB153), which increased with increasing total lipids content because of N starvation (Halling-Sørensen et al., 2000). The $logBAF_{lip}$ of *P. donghaiense* for the PBDEs also increased with increasing total lipid content because of the variation in N concentration (**Fig. 5a**). However, the $BAFs_{lip}$ under 0 μmol P/L was higher than that under 8 μmol P/L but lower than that under 32 μmol P/L (**Table 1**). The BAF_{lip} under different P concentrations did not increase with increasing total lipids content (**Fig. 5b**). This phenomenon was also observed in the BCF of *S. carpricornutum* for 2,3,3′,4,4′-pentachlorobiphenyl (PCB105) under N starvation (Halling-Sørensen et al., 2000), which was attributed to the structure and polarity of the hydrophobic molecules and the composition of the algal lipids. Algal lipids consist of neutral lipids and polar lipids, including glycolipids and phospholipids (Deng et al., 2011). Many studies have reported that N deficiency induced a significant increase in the neutral lipids content of phytoplankton (Illman et al., 2000; Merzlyak et al., 2007). Meanwhile, reports indicated that the phospholipids of *Monodus subterraneus* clearly decreased, but the neutral lipids markedly increased when phosphate was absent in the media (Khozin-Goldberg and Cohen, 2006). On the other hand, Reitan et al. (1994) reported that P deficiency decreased the neutral lipids content of *Nannochloris atomus* and *Tetraselmis* sp. Halling-Sørensen et al. (2000) found differences among the total lipid-normalized BCFs, the neutral lipid-normalized, and the polar lipid-normalized BCFs. Therefore, the $BAFs_{lip}$ of *P. donghaiense* for PBDEs may also be influenced by the composition of algal lipids, which should be further studied in the future.

Lipid-normalized BAFs for microalgae were assumed directly correlated to the octanol/water partition coefficient (K_{ow}) of the compound (Connolly and Pederson, 1988). Previous studies reported that the logBAF of hydrophobic organic compounds with $\log K_{ow} < 6$ was a linear function of $\log K_{ow}$ with a slope of 1, whereas the logBAF–$\log K_{ow}$ relationship at $\log K_{ow} > 6$ could take non-linear shapes such as a level off or decrease (Swackhamer and Skoglund, 1993; Stange and Swackhamer, 1994; Gerofke et al., 2005). One possible reason for the non-linearity of the logBAF–$\log K_{ow}$ relationship was the slow uptake rate of chemicals with higher molecular volume or higher K_{ow} by the microalgae (Seto and Handoh, 2009). Furthermore, the logBAF–$\log K_{ow}$ relationship was also influenced by the growth dilution of microalgae. Swackhamer and Skoglund (1993) reported that PCBs with $\log K_{ow} < 6.3$ presented a linear logBCF when the microalgae were slowly growing. Meanwhile, PCBs with $\log K_{ow} < 5.5$ were obtained when the microalgae were actively growing. In addition, the logBAF–$\log K_{ow}$ relationship is affected by species-specific differences in phytoplankton, which may result from their lipids compositions (Stange and Swackhamer, 1994). The $\log K_{ow}$ of BDE28, BDE47, and BDE99 were 5.94, 6.81, and 7.32, respectively (Braekevelt et al., 2003; Wania and Dugani, 2003; Guan et al., 2009). Although the nutrient concentrations affected the BAFs of *P. donghaiense* for PBDEs to some extent, the BDE28 $logBAF_{lip}$ was close to its $\log K_{ow}$. However, those of BDE47 and BDE99 were clearly lower than their respective $\log K_{ow}$ (**Fig. 6**). This relationship is similar to that in previous study (Stange and Swackhamer, 1994; Seto and Handoh, 2009). In addition, Swackhamer and Skoglund (1993) found that the logBAFs of microalgae for PCBs with $\log K_{ow}$ from 5.2 to 6.0 were between 5 and 5.5 after 24 hr of exposure. In the present study, the logBAFs for BDE28 ($\log K_{ow} = 5.94$) under different N and P concentrations was between 5.41 and 5.93, which indicated that our BAFs was corresponding with those of other studies.

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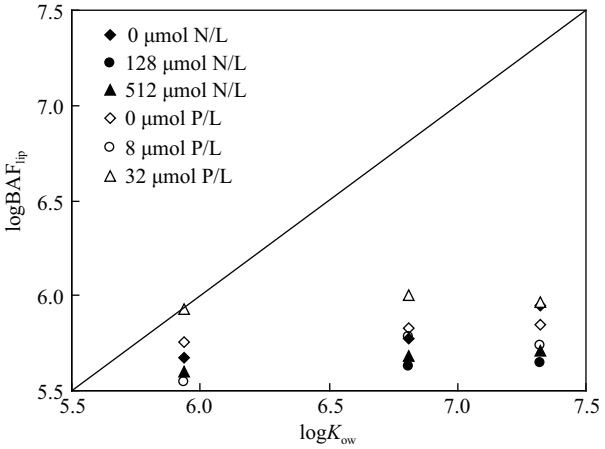

Fig. 6 Relationship between the logBAF$_{lip}$ for PBDEs of *P. donghaiense* and its logK_{ow} under different N and P concentrations. The line is reference line whose slope is 1.

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