Bioresource Technology 177 (2015) 289–297

Contents lists available at ScienceDirect

Bioresource Technology

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

Isolation of microalgae tolerant to polybrominated diphenyl ethers (PBDEs) from wastewater treatment plants and their removal ability

Dan Deng [a], Nora Fung-yee Tam [a,b,*]

[a] Department of Biology & Chemistry, City University of Hong Kong, Tat Chee Avenue, Kowloon Tong, Kowloon, Hong Kong Special Administrative Region
[b] State Key Laboratory in Marine Pollution, City University of Hong Kong, Tat Chee Avenue, Kowloon Tong, Kowloon, Hong Kong Special Administrative Region

HIGHLIGHTS

- Nine microalgal strains were isolated from influents of WWTPs spiked with PBDEs.
- The isolates were identified based on the morphological and 18S rDNA analysis.
- The tolerance and removal ability of different isolates to PBDEs were compared.
- A *Chlorella* isolate with high tolerance to PBDEs and removal ability was obtained.
- Bioaccumulation and biotransformation were important for PBDE removal.

ARTICLE INFO

Article history:
Received 17 October 2014
Received in revised form 23 November 2014
Accepted 25 November 2014
Available online 2 December 2014

Keywords:
PBDEs
Microalgal isolate
Resistance
Removal
Wastewater treatment plant

ABSTRACT

The present study isolated microalgae with tolerance to polybrominated diphenyl ethers (PBDEs) from wastewater aiming to discover isolates with high removal abilities. Nine isolates, *Chlorella* (STCh and SICh), *Parachlorella* (STPa1 and STPa2), *Scenedesmus* (STSc, TPSc1 and TPSc2), *Nitzschia palea* (YLBa) and *Mychonastes* (TPMy), were obtained. Four isolates, SICh, STCh, STPa1 and TPSc1, were very tolerant, and their growth was not affected by DE-71 and BDE-209 mixtures (5:1) at low (6 $\mu g\ L^{-1}$), medium (60 $\mu g\ L^{-1}$) or even high (600 $\mu g\ L^{-1}$) levels for 7 days. The removal of PBDEs by one of the tolerant isolates, SICh, was the highest, with 82–90% removal at the end of 7-days exposure. SICh also accumulated more PBDEs than the other isolates. Bioaccumulation and biotransformation were important for PBDE removal. This is the first study isolated PBDE-tolerant microalgae from wastewater and obtained a *Chlorella* isolate, SICh, with high tolerance and removal ability.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) commonly used as brominated flame retardants in household items and industrial products are potentially leaching out into surrounding environments during their production, usage and disposal. PBDEs, due to their physical and chemical properties, are persistent, bioaccumulative and toxic to environments. Although PBDEs have been gradually phased out in many countries, old products containing significant quantities of PBDEs may still release these pollutants into the environment. PBDEs have been detected in both influent and effluent of wastewater treatment plants (WWTPs), indicating that conventional treatment processes based on sedimentation and microorganisms dominated by bacteria are not effective in removing PBDEs (Deng et al., 2015). Microalgae have been suggested as an alternative biological process for the removal of toxic organic pollutants from domestic and industrial wastewaters such as nonylphenol (NP) (Gao et al., 2011), polycyclic aromatic hydrocarbons (PAHs) (Lei et al., 2007) and polychlorinated biphenyls (PCBs) (Fitzgerald and Steuer, 2006). The latter persistent organic pollutants (POPs) have similar chemical structure as PBDEs, but the ability of microalgae to remove and degrade PBDEs has never been reported.

It has been suggested that indigenous microorganisms found in industrial wastewater or contaminated water could be more adaptive to the environment and more tolerant to pollutants than the commercial and laboratory species (Ahemad and Malik, 2011). An ecotype green alga *Stigeoclonium tenue* isolated from ditches containing mining water was tolerant to zinc (Zn), while another ecotype of the same species isolated from unpolluted lake water was Zn-sensitive (Pawlik-Skowrońska, 2003). The isolates obtained

* Corresponding author at: Department of Biology & Chemistry, City University of Hong Kong, Tat Chee Avenue, Kowloon Tong, Kowloon, Hong Kong Special Administrative Region.
E-mail address: bhntam@cityu.edu.hk (N. F.-y. Tam).

http://dx.doi.org/10.1016/j.biortech.2014.11.103
0960-8524/

from contaminated environments could also display higher removal abilities than those obtained from clean and pristine environments (Guo et al., 2005). Chong et al. (2000) reported that a green microalga, *Chlorella miniata* isolated from wastewater treatment plants was more efficient in removing nickel (Ni) and Zn than the commercial *Chlorella vulgaris*. In the past decades, different species of microalgae belonged to the genera of *Chlorella*, *Scenedesmus* and *Asterionella* were isolated from contaminated waters for the bioremediation of pollutants such as PCBs (Cosper et al., 1988). It is therefore logical to hypothesize that the sewage from WWTPs with PBDE contamination is a potential source of PBDE-tolerant microalgal species and the tolerant isolates may be more effective in removing PBDEs.

The present study aims to (i) obtain PBDE-tolerant microalgae from influent of WWTPs using the standard isolation technique, and identify them based on their morphology and molecular properties; (ii) evaluate the tolerance of different isolates to PBDEs following the standard toxicity assessment test; (iii) compare the ability of different isolates to remove PBDEs and discover the isolate with the highest removal ability; and (iv) elucidate the relationships between tolerance and removal abilities of microalgal isolates.

2. Methods

2.1. Collection of influent from wastewater treatment plants

Primary settled influent from four different WWTPs in Hong Kong, namely Shatin, Stonecutters Island, Tai Po and Yuen Long were collected. The first two WWTPs, Shatin and Stonecutter Island, receive mainly municipal sewage with daily inflows of 225×10^3 and 1385×10^3 m^3, respectively, while the latter two (Tai Po and Yuen Long) receive both municipal and industrial wastewaters, with respective daily inflows of 96×10^3 and 18×10^3 m^3 (Data provided by Hong Kong Drainage Services Department). The concentrations of PBDEs in these four WWTPs ranged from 3 to 89 ng L^{-1}, 15 to 124 ng L^{-1}, 1 to 254 ng L^{-1}, 12 to 233 ng L^{-1}, respectively (Deng et al., 2015). Influent from three WWTPs, Shatin, Stonecutter Island and Tai Po had salinities ranging from 8 to 12 parts per thousands (‰) as seawater is used for toilet flushing in these districts. The only influent with freshwater salinities (around 0.6‰) was Yuen Long WWTP that uses freshwater for toilet flushing.

2.2. Isolation and identification of PBDE-tolerant microalgal species

The influent was filtered through a 100 μm mesh net immediately after collection. The filtered influent was spiked with a mixture of commercial penta-BDE product (DE-71) (Wellington laboratories, Canada) and BDE-209 (J&K Scientific, China) (5:1) dissolved in dimethyl sulfoxide (DMSO) at a total concentration of 6 μg L^{-1}. The influent with 0.1% DMSO but no PBDEs was served as the control to understand the microalgal diversity in influent. The preliminary experiments showed that 0.1% DMSO did not have any significant effects on the growth of microalgae in influent (data not shown). The spiked influent and control were incubated at 22 ± 1 °C on a rotary shaker with a speed of 150 rpm, illuminated with cold fluorescent lamps at a light intensity of 40 μmol s^{-1} m^{-2} and a 16/8 h light/dark cycle in an environmental chamber. After 2 weeks, the enriched microalgal communities were observed under a light microscope (Zeiss, Axioskop, Germany) at a magnification of 400×, and were identified based on their morphology (Bellinger and Sigee, 2010). Species found in the PBDE-spiked influent were considered as the tolerant isolates which were further isolated to obtain the axenic culture for comparison of their tolerance and removal abilities. On the other hand, the strains only present in the control but not in the PBDE-spiked influent were the sensitive ones which were not further explored in the present study.

To obtain the axenic culture of the tolerant isolate, 1 mL of the enriched culture from the PBDE-spiked influent was serially diluted and inoculated on the agar plates containing Bristol medium (BM) (James et al., 1978) or Chu No. 10 medium (for diatom) (Chu, 1942). The plates were incubated under the same condition as described above. After 2 weeks, the visible microalgal colonies with distinct morphology were picked and sub-cultured for the next round of selection until an axenic culture was obtained. A total of nine tolerant cultivable isolates were obtained. The morphology of each isolated species or strain was observed by microscopy as described above. 1 mL of the cultivable cells at the exponential phase was harvested by centrifugation at 10,000 rpm for 1 min, and the genomic DNA of the cell pellet was extracted using the PowerSoil DNA Isolation Kit (MO BIO Laboratories, Inc., USA). Two pairs of oligonucleotide primers prepared by Life Technologies were used for the amplification of microalgal 18S rDNA sequences. Forward 1 (GTCAGAGGTGAAATTCTTGGATTTA) and reverse 1 (AGGGCAGGGACGTAATCAACG) were universal eukaryotic primers (Rasoul-Amini et al., 2009), while forward 2 (GCGTAAATCCCGACTTCTGGAAGGG) and reverse 2 (ATGCCCCCGACTGTCCCTCTTAATC) were designed from multiple alignment of the known 18S rDNA sequences of the family Chlorellaceae. The PCR amplification products were sequenced by Beijing Genomics Institute (BGI, China), and the sequences were compared with those in the GenBank database by Blast. The sequences of most related species were imported to ClastalW for alignment and the phylogenic relationships were analyzed by MEGA version 5.1.

2.3. Toxicity assessment

Cells of each isolate obtained from PBDE-spiked influent were grown in 250 mL Erlenmeyer flasks containing 100 mL sterilized BM on a rotary shaker at 150 rpm for 1 week to reach an exponential growth phase. Cells were harvested by centrifugation at 4000 rpm for 15 min, washed with BM and re-suspended in BM spiked with a mixture of DE-71 and BDE-209 (5:1) at three contamination levels with an initial biomass of 30 mg dry weight L^{-1} medium. This initial cell biomass was chosen to have sufficient viable cells and have the maximum sensitivity to PBDEs (Monteiro et al., 2011). The three levels of PBDE mixture were 6 (low), 60 (medium) and 600 (high) μg L^{-1}, each in triplicate. Cells grown in 0.1% DMSO BM without PBDEs were served as the solvent control to compare the effects of PBDE on the growth of microalgae. Preliminary experiments showed that the solvent DMSO did not have any significant effects on the growth of all microalgal isolates.

On days 1, 3, 5 and 7, flasks were retrieved and the cultures were harvested. Growth, in terms of cell number, chlorophyll (*Chl*) a content and dry weight at 105 °C, were determined according to the standard methods described by Gao et al. (2011). The *Chl*-specific growth rate (μ) and doubling time (*Td*) at the exponential phase, as well as the percentages of growth inhibition (*GI*), at different levels of PBDEs were calculated as follows:

$$\mu(\text{day}^{-1}) = (\ln\ Chl_n - \ln\ Chl_0)/t_n$$

$$Td(\text{day}) = \ln\ 2/\mu$$

$$GI_{\mu_i}(\%) = (\mu_c - \mu_i) \times 100/\mu_c$$

where Chl_n and Chl_0 are *Chl* a concentrations at time t_n and t_0 (initial), respectively, while μ_c and μ_i are growth rates of the control and treated group *i*, respectively.