



# A coupled UV photolysis-biodegradation process for the treatment of decabrominated diphenyl ethers in an aerobic novel bioslurry reactor

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## Abstract

The commercial flame retardant is an emerging contaminant (EC) commonly found in soils and sediments. A coupled UV-photolysis-biodegradation process was used to decompose decabromodiphenyl ether (BDE-209) in clay slurries. A novel bioslurry bioreactor (NBB) was employed in which BDE-209 degradation was maximized by the simultaneous application of LED UVA irradiation and biodegradation by a mixed bacterial culture. The rate of BDE-209 degradation decreased in the order: coupled UV photolysis-biodegradation ( $1.31 \times 10^{-2} \text{ day}^{-1}$ ) > UV photolysis alone ( $1.10 \times 10^{-2} \text{ day}^{-1}$ ) > biodegradation alone ( $1.00 \times 10^{-2} \text{ day}^{-1}$ ). Degradation intermediates detected included hydroxylated polybrominated diphenylethers, partially debrominated PBDE congeners and polybrominated dibenzofuran. The UV-resistant bacterial strains isolated that could utilize BDE-209 as a sole carbon source included *Stenotrophomonas* sp., *Pseudomonas* sp., and *Microbacterium* sp. These strains encoded important functional genes such as dioxygenase and reductive dehalogenases. Continuous UV irradiation during the NBB process affected various biochemical oxidative reactions during PBDEs biodegradation. Simultaneous photolysis and biodegradation in the NBB system described reduces operational time, energy, expense, and maintenance-demands required for the remediation of BDE-209 when compared to sequential UV-biodegradation process or to biodegradation alone.

**Keywords** Coupled UV photolysis-biodegradation · Decabromodiphenyl ether · Novel bioslurry bioreactor · UV-resistant bacterial strains · Polybrominated dibenzofuran

## Highlights

- A time, energy, and cost-saving technique using a coupled UV photolysis-biodegradation process for degrading BDE-209 in a novel bioslurry bioreactor.
- Identification of isolated UV-resistant bacterial strains that potentially are able to utilize PBDEs as sole carbon source and the analysis of related functional genes.
- The prediction of a possible pathway for BDE-209 degradation by coupled photolysis-biodegradation.

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## Introduction

Polybromodiphenyl ethers (PBDEs) have been widely used as brominated flame-retardants in many commercial products including textiles, furniture, and electronics. Decabromodiphenyl ether (BDE-209) is the most prevalent congener in commercial PBDE mixtures. This congener was targeted in the elimination annex of the 2017 Stockholm Convention. Because BDE-209 is resistant to environmental degradation via chemical, biological, and photolytic processes, it is considered a persistent organic pollutant (POP). It is released into the environment during production, use, storage, disposal, and recycling, and has become one of the most commonly encountered emerging contaminants (ECs) (McGrath et al. 2017). Soil contamination by PBDEs seems especially prevalent in China and other developing countries. For example, high concentrations of BDE-209 in soils and sediments are encountered at many electronic waste disposal sites in China, including those within the Pearl River Delta region in Guangdong Province, and in industrial areas where BDE-209 is manufactured, as for example Laizhou Bay

in Shandong Province (Ji et al. 2017). Five electronic waste disposal sites in Guangdong Province were surveyed and found to have BDE-209 concentrations in soil of approximately  $200 \text{ ng g}^{-1}$ , and four river sediments in Shandong Province were found to contain BDE-209 at levels above  $200 \text{ ng g}^{-1}$ . A soil sample from Shandong Province contained the highest level of BDE-209, viz.  $1,852 \text{ ng g}^{-1}$ .

The toxic effects of BDE-209, which may be manifested in both humans and wildlife, include immunotoxicity, cytotoxicity, neurotoxicity, genotoxicity, mutagenicity, carcinogenicity, and teratogenicity (Segev et al. 2009). Furthermore, PBDEs congeners formed from BDE-209 debromination, such as tetra-BDEs, hepta-BDEs, and bromophenols, have been identified as endocrine disrupting chemicals (EDCs) that adversely affect thyroid hormone regulation, neurodevelopment, and reproductive health in certain animals (Liu et al. 2011). The fathead minnow (*Pimephales promelas*) exposed to a low dose of BDE-209 ( $3 \text{ ng g}^{-1} \text{ bw-day}$ ) for 28 days showed a 53% and 46% decline in total circulating thyroxine (TT4) and 3,5,3'-triiodothyronine (TT3), respectively, while TT4 and TT3 deficits at a higher dose of BDE-209 ( $300 \text{ ng g}^{-1} \text{ bw-day}$ ) were 59% and 62%, respectively. Brain deiodinase activity (T4-ORD) was reduced by about 65% at both the above doses (Noyes et al. 2013). Exposure of a mammalian (male rat) model to BDE-209 resulted in disruption of thyroid function leading to hypothyroidism; in this case, the underlying mechanisms seemed to involve oxidative stress and perturbations of hypothalamic-pituitary-thyroid axis (Wang et al. 2019). Clearly, treatment systems that achieve complete bio-mineralization of BDE-209 are needed to eliminate the adverse effects of BDE-209 on human and ecosystem health.

A number of treatment systems involving coupled photolysis and biological processes have been developed with the goal of enhancing the removal of refractory aromatic compounds from industrial wastewater. For example,  $200 \text{ mg L}^{-1}$  of Reactive Black 5 (RB5) azo dye A in textile wastewater was shown to be degraded by such a coupled process  $\text{TiO}_2$  and 247.3-nm UVC irradiation and aerobic biodegradation (Shan 2013). Cleavage of aromatic rings in RB5 occurred to a significantly degree after 2 h of illumination, and approximately 60% COD removal was achieved during aerobic biodegradation by a single strain of *Pseudomonas aeruginosa*. Similarly, benzotriazole decomposition was achieved using a coupled process consisting of 254-nm UV irradiation (light intensity of  $2.12 \text{ mW cm}^{-1}$ ) and aerobic biodegradation by an enrichment of benzotriazole-degrading bacteria; a rate constant of  $1.39 (\mu\text{m mol L}^{-1})^{0.34} \text{ h}^{-1}$  was reported (Shan et al. 2017). In contrast to these examples of contaminant removal from wastewater, few studies have focused on using coupled photolysis-biodegradation process for the degradation of POPs/ECs present in soil slurries.

Aerobic bioslurry reactors (ABRs) have been used to remediate POPs in contaminated soil/water slurries. The effective removal of POPs is achieved when complete mixing occurs in an ABR; under such circumstances, PAHs and similar aromatic chemicals have been shown to be biodegraded successfully (Yu et al. 2019a). However, the cleanup of PBDE-contaminated soil/sediment using ABRs presents difficult reactor design challenges if the requirements are rapid remediation times and low cost. An alternative process for remediating PBDE-contaminated soils is proposed in this study, namely the simultaneous coupling of UV photolysis and biodegradation. As a first step toward our goal of treating contaminated soils, montmorillonite clay was selected for study since clays are major component geosorbents found in most soils. A novel bioslurry bioreactor (NBB) that uses coupled UV photolysis and biodegradation is proposed to accelerate BDE-209 removal. UV-resistant bacteria capable of degrading BDE-209 were used in this NBB system. We quantified the major organic decomposition intermediates, as well as the bromide anion, present during the biodegradation of BDE-209 by this coupled photolysis-biodegradation process. The bacterial strains present were evaluated to determine if they are able to utilize PBDEs as a sole carbon source. The RNA expression levels of specific functional genes involved in BDE-209 degradation were measured using PCR and specific primers; these included various ring-cleavage dioxygenases and dehalogenases. Finally, a pathway for BDE-209 degradation in the NBB system is proposed.

## Materials and methods

### Chemicals

BDE-209 with a purity greater than 98% (Alfa aesar, Germany, 99% purity) was used to prepare the BDE-209-contaminated sediment, and as the sole carbon substrate when assessing the status of the BDE-209-biodegrading bacteria. A standard solution of BDE-209, dissolved in isooctane-toluene (8:2, v/v), was used for the GC/MS analysis. A standard solution consisting of 38 PBDEs (Cambridge Isotope Laboratories, Inc., USA, 99% purity) and BDE-15 (Merck, Germany, 99% purity) was used for the bacterial utilization analysis. All organic solvents, including n-hexane and acetone, were of GC grade with a purity of > 99.9%. All other chemicals were reagent grade with a purity of > 99%. The Milli-Q water (>  $18 \text{ M}\Omega\text{cm}$ ) used in this study was obtained from a Millipore water purification system.

### Sorbent and BDE-209-biodegrading bacteria

Ca-montmorillonite (clay) was selected as a representative soil geosorbent for use in the sorbent/water slurry system. The clay

was used to eliminate the confounding influence of soil organic matter (SOM), present in surface soils and to a much lesser extent in subsoils on UV photolysis and biodegradation. Table S1 shows the physical-chemical characteristics of clay, which was purchased from the Clay Minerals Society, Purdue University, USA. The UV-resistant mixed bacterial culture came from a PBDEs-contaminated sediment collected from the Da-An River, Taiwan; this culture has previously been identified as having the ability to biodegrade BDE-209 and other PBDEs (Chang et al. 2019).

### Design of the NBB

The NBB was designed to carry out an integrated process that simultaneously combined a UV-resistant mixed bacterial culture that was able to utilize BDE-209 as sole carbon source with UV photolysis. Continuous UV radiation is able to generate significant numbers of free radicals and these are able to breakdown the chemical structure of BDE-209 and related other intermediates. BDE-209 biodegradation can also occur at the same time. Figure 1 shows the NBB design that combines UV photolysis with a biological process for the treatment of ECs. The NBB was modified from an original bioreactor that had a sequential UV photolysis and biological processes for BDE-209 treatment and had been used in our previous study (Chang et al. 2020). The NBB consists of two reactors made up of different materials, namely a glass outer reactor and a quartz inner tank. The volume of the inner tank and the outer tank was 4924 cm<sup>3</sup> and 330 cm<sup>3</sup>, respectively. A number (72) of 365-nm LED-UV light lamps (45 × 45 mil HI-POWER UV-LED CHIP, Advanced Optoelectronic Technology Inc., Taiwan) were fixed onto four aluminum-made cooling fins and these were distributed evenly within the inner tank. UV light is able to easily penetrate the quartz wall and enter the clay/water system. The energy intensity of UV irradiation at the outer surface of quartz inner tank was measured and found to be in the range 0.58 to 0.97 mw cm<sup>-2</sup>. The clay/water system was added separately into the space between inter tank and outer tank, which has an effective volume of 2598 mL. An overhead mixer with an PTFE stirrer was set up in the tank to allow complete mixing and to promote aerobic conditions.

### The coupled photolysis-biological process for BDE-209 treatment within the NBB

A coupled UV photolysis-biodegradation process for BDE-209 treatment was initiated by adding the Da-An bacterial community into BDE-209-contaminated clay/water system inside the NBB. Complete mixing was achieved using a 280-rpm stirring rate for 140 days; during this time, LED UVA lights were used to provide a steady energy intensity. The method of creating the BDE-209-contaminated clay/

water system was such that it resulted in clay theoretical containing 25 mg kg<sup>-1</sup> BDE-209 in mineral salt basal (MSB); this procedure was described in our previous study (Chang et al. 2020). The ratio of the clay/water system was designed to give a ratio of 2 g:25 mL. The experiments consisted of the following: (1) coupled UV photolysis-biodegradation; (2) UV photolysis alone; and (3) biodegradation alone in an absolutely dark environment. Two controls were included; these were the no treatment control (control 0) and biodegradation using a culture sterilized with 1% NaN<sub>3</sub> (control 1). The pH values and ORP values of the slurries were measured and averaged 7.32 ± 0.21 (the range of 7.22–7.52) and 218 ± 26 mV (the range of 188–244 mV), respectively, during experiments.

### Analytical methods

#### BDE-209 concentration analysis and metabolites identification

A detail procedure of BDE-209/metabolites analysis in the bioslurry was carried out and this was based on the one used in our previous study (Chou et al. 2016). Ultrasound-assisted extraction from the bioslurry was selected as sample pretreatment when carrying out GC analysis of BDE-209. The concentration of BDE-209 was measured by GC using a pulsed discharge electron capture detector. Possible intermediates detected using the GC/MS full scan mode were identified by prediction, but were not quantified. The m/z profiles of the metabolites were identified using the GC/MS spectra databases in the NIST 17 library.

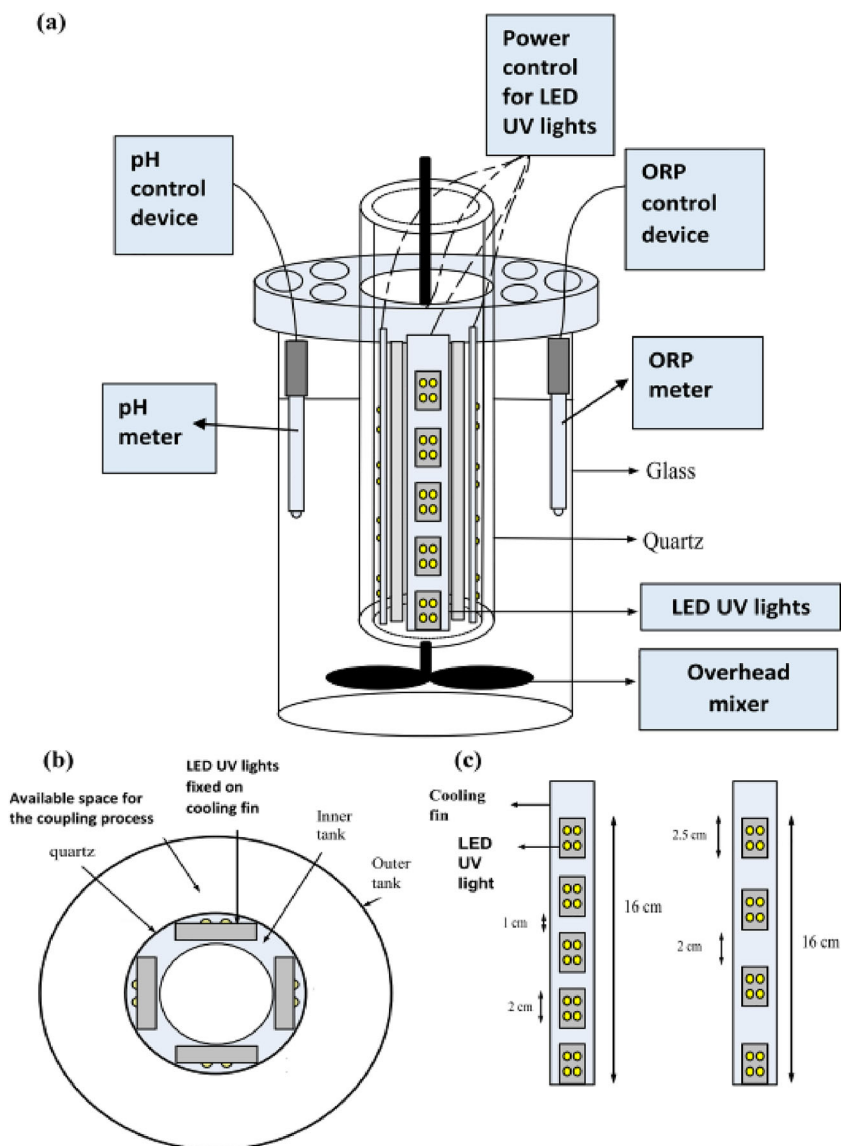
#### Anion analysis

Bromide (Br<sup>-</sup>) was measured during the coupled BDE-209 UV photolysis/biodegradation process by ion chromatography (Metrohm 883, Switzerland) using an autosampler (Metrohm 863 Switzerland), and a conductivity detector. Samples of supernatant liquid were separated by high-speed centrifugation, filtered through a 0.22-μm nylon filter, and then measured immediately. The retention times within the ion chromatographic profiles were 8.29 min.

#### Bacterial strains and their utilization of PBDEs as sole carbon resource

Bacterial strains capable of using BDE-209 or other PBDEs as sole carbon source were isolated during BDE-209 biodegradation in the clay/water system. To do this, 10 mL of the sorbent/water mixture on day 43, 74, 105, and 140 was collected and subjected to 10-fold serial dilution. Next 0.2 μL of inoculum was spread on purified agar plates made containing 20.0 μg L<sup>-1</sup> BDE-209 as sole carbon source plus MSB. To avoid bacterial strains that are able to utilize the organic

**Fig. 1** Schematic diagram of the NBB used for coupled UV photolysis-biodegradation: **a** the design of the NBB; **b** a top-down view; **c** the design of the 365-nm LED UV-lights, which are fixed to the cooling fines



residues present in agar as a carbon source, purified agar was obtained by washing the agar powder with Milli-Q water at least three times before medium preparation. Pure stains, as single colonies, were identified after incubation at 25 °C for 14 days. These isolated strains were confirmed as being able to grow well on BDE-209-MSB agar plates by the streak method technique and this streaking was repeated at least 3–5 times. Finally, these strains were identified by Mission Biotech Company (Taiwan) based on the V1-V8 domain sequences of their 16S rDNA regions.

A Biolog MT2 microplate (Hayward, CA, USA)-based assay was used for the rapid identification and evaluation of the ability of a given bacterial strain to utilize PBDEs as a sole carbon substrate. Each Biolog MT2 microplate well was created as a sole carbon source substrate well using MSB; in addition, an equal concentration of tetrazolium violet dye,

which is sensitive to the oxidation of a carbon source and to bacterial respiration, was added (Taha et al. 2015; Chang et al. 2020). Different types of PBDEs, namely BDE-209 (25.0 µg L<sup>-1</sup>), BDE-15 (25.0 µg L<sup>-1</sup>), and a mixture of 38 PBDE congeners, including mono-BDE, di-BDE, tri-BDE, tetra-BDE, penta-BDE, hexa-BDE, and hepta-BDE were selected as the carbon sources. Table S2 shows the concentrations of mixed PBDE congeners used in the Biolog MT microplates and these had the range 5.0–12.5 µg L<sup>-1</sup>. The microplate wells were then inoculated with 150 µL of each isolated bacterial strain, which gave a bacterial density or OD<sub>590</sub> of between 0.2 and 0.3, 10<sup>7</sup> CFU mL<sup>-1</sup> on R2A agar; the incubation was carried out at 30 °C in the dark for 48 h. Positive and negative controls for bacterial respiration, using the Biolog MT2 microplate wells, were set up for each isolated strain and contained 25.0 µg L<sup>-1</sup> glucose-MSB and MSB, respectively. The OD value of each



microplate well was measured using an ELISA Reader at 590 nm. A positive response, namely the ability to carry out BDE-209 biodegradation, was assessed using the equation (Sample  $OD_{590}$  – Negative control  $OD_{590}$ ) and this involved measuring the purple color present in the Biolog MT2 wells. Four levels of BDE-209 utilization were defined based on the relative  $OD_{590}$  values (see above) obtained in this study; these were very strong ( $\geq 0.4$ ), strong (0.3–0.4), medium (0.1–0.3), and weak ( $< 0.1$ ).

### Detection of genes in the isolated identified bacteria encoding functional enzymes related to BDE-209 biodegradation

Genomic DNA from each strain was assessed in order to detect the presence of genes encoding functional enzymes related to BDE-209 biodegradation. Table 1 lists the published primer pairs that were used to detect the following functional genes; these genes are likely to be involved in BDE-209 biodegradation. One target, dioxygenases, are able to carry out benzene ring-cleavage reactions under aerobic condition; these enzymes include catechol 2,3-dioxygenases (EC 1.3.11.2, C23O), aromatic ring hydroxylating dioxygenase (ARHD), and rieske iron-sulfur dioxygenases (Rf). In addition, reductive dehalogenases that carry out biological debromination under aerobic condition were also screened for, namely Rdha and Rdase. TCEse and PceA were used as the negative control of biological debromination under strict

anaerobic condition. The PCR reagents used for the reaction were 2  $\mu$ L purified genomic DNA, 1.5  $\mu$ L each of the primers (10  $\mu$ M), and 5  $\mu$ L of the 5  $\times$  PCR master mix in the kit (Gene Mark, Taiwan); these were added to a tube together with 15  $\mu$ L of sterilized Milli-Q water. Finally, the PCR amplification products were identified by 1% agarose gel electrophoresis.

## Results

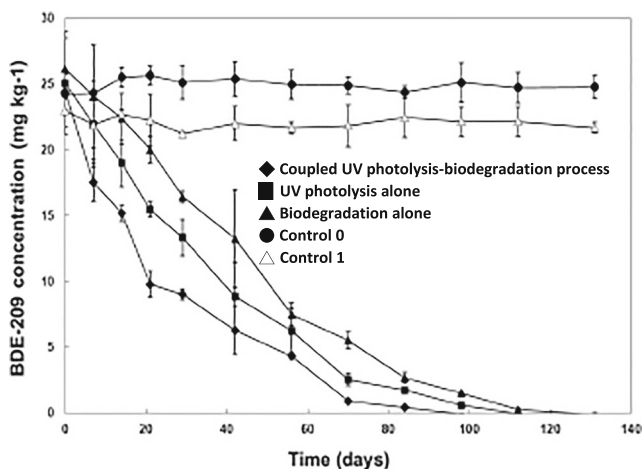
### BDE-209 concentration and bacterial numbers in the NBB

Degradation of BDE-209 in the NBB over time is shown in Fig. 2. The coupled UV photolysis-biological process removed BDE-209 effectively. The concentration of BDE-209 decreased from  $24.43 \pm 1.33$  mg  $kg^{-1}$  to  $0.43 \pm 0.07$  mg  $kg^{-1}$  over 84 days using the coupled process. In comparison, the rate of BDE-209 degradation by the UV photolysis process alone was reduced compared to the coupled process, but faster than biodegradation alone. Specifically, the concentration of BDE-209 decreased from  $25.09 \pm 3.95$  mg  $kg^{-1}$  to  $0.06 \pm 0.02$  mg  $kg^{-1}$  after 98 days using UV photolysis alone, while the concentration of BDE-209 decreased from  $26.17 \pm 2.32$  mg  $kg^{-1}$  to  $0.27 \pm 0.09$  mg  $kg^{-1}$  after 112 days using biodegradation alone. Previously, the biodegradation kinetics of BDE-209 using a clay/water system was shown to be first-order (Chou et al. 2016). The first-order rate constant ( $k$ ) for

**Table 1** The primers used for DNA amplification of the various function genes present during BDE-209 degradation

No.	Functional genes (abbreviation)	Primers	Primer sequence (5' → 3') <sup>a</sup>	Position (bp)	PCR annealing temp. (°C)	References
1	Aromatic ring hydroxylating dioxygenases (C23O)	C23Or C23Of	CCAGCAAACACCTCGTTGCGGTTGCC AAGAGGCATGGGGGCGCACC GGTTCGATCA	450	53	Alfreider et al. 2003
2	Aromatic ring hydroxylating dioxygenases (ARHD)	888f 300r	TGCASSTWTCACGGSTGG CTCGACTCCGAGCTTCCAGTT	340	49	Kitagawa et al. 2001
3	Rieske iron-sulfur motif (Rf)	Rf1 Rr1	AGGG ATCCCCANCCRTGRTANSWRCA TGTTCCCGAAGCTTGCTTC	700	55	Kumar et al. 2005
4	<i>rdh A</i> dehalogenation (RdhA)	RRf2 B1r	SHMGBMGWGATTTYATGAARR CHADHAGCCAYTCRTACCA	1,500–1,700	49	Krajmalnik-Brown et al. 2004
5	Reductive dehalogenase for dehalogenation of <i>Dehalobacter</i> (RDase)	179f 1007r	TGTATTGTCCGAGAGGCA ACTCCCATATCTCTACGG	830	53	Schlötelburg et al. 2002
6	TCE dehalogenation of <i>Dehalococcoides</i> (TCEse)	TCEsef TCEser	GGTAATACGTAGGAAGCAAGCG CCGGTTAAGCCGGGAAATT	1,400	60	Holmes et al. 2006
7	<i>pce A</i> dehalogenation (PceA)	SpDr1f SpDr1r	CGTTGGACCTATTCCACCTG CAAGAACGAAGGCAATCACA	199	53	Suyama et al. 2002

a: Degenerate nucleotides positions: R = A or G; K = G or T; M = A or C; S = C or G; W = A or T; Y = C or T; B = C, G or T; D = A, G or T; V = A, C or G; H = A, C or T



**Fig. 2** BDE-209 degradation by coupled UV photolysis-biodegradation in an NBB. Control 0: no treatment; Control 1: biodegradation process only treated with 1% NaN<sub>3</sub> (sterilization)

BDE-209 degradation was found to be  $9.3 \times 10^{-3} \text{ day}^{-1}$  for the Da-An bacterial-mixed culture. The first-order-rate constants for BDE-209 degradation in the present study decreased in the order coupled UV photolysis-biodegradation process ( $1.31 \times 10^{-2} \text{ day}^{-1}$ ,  $r^2 = 0.9972$ ) > UV photolysis alone ( $1.10 \times 10^{-2} \text{ day}^{-1}$ ,  $r^2 = 0.9952$ ) > biodegradation alone ( $1.00 \times 10^{-2} \text{ day}^{-1}$ ,  $r^2 = 0.9684$ ).

Changes in bacterial populations over time were estimated by plating samples on agar containing BDE-209 as sole carbon; samples from the NBB obtained during the coupled UV photolysis-biodegradation process and biodegradation alone were compared in Fig. 3. The average number of bacteria present during the coupled UV photolysis-biodegradation process was lower than during the biodegradation process alone. The number of bacteria present during the coupled UV photolysis-biodegradation process decreased from  $1 \times 10^7 \text{ CFU mL}^{-1}$  on day 0 to  $3.8 \times 10^4 \text{ CFU mL}^{-1}$  on day 28. The number of bacteria present then increased to a high of  $2.6 \times 10^6 \text{ CFU mL}^{-1}$  on day 101, which was followed a second decrease to  $7.8 \times 10^4 \text{ CFU mL}^{-1}$  on day 120. A similar trend was found for biodegradation alone, which decreased from  $1 \times 10^7 \text{ CFU mL}^{-1}$  on day 0 to a low of  $1.8 \times 10^5 \text{ CFU mL}^{-1}$  on day 42, followed by a maximum of  $8.9 \times 10^6 \text{ CFU mL}^{-1}$  on day 72 and a second decrease to  $2.1 \times 10^6 \text{ CFU mL}^{-1}$  on day 120.

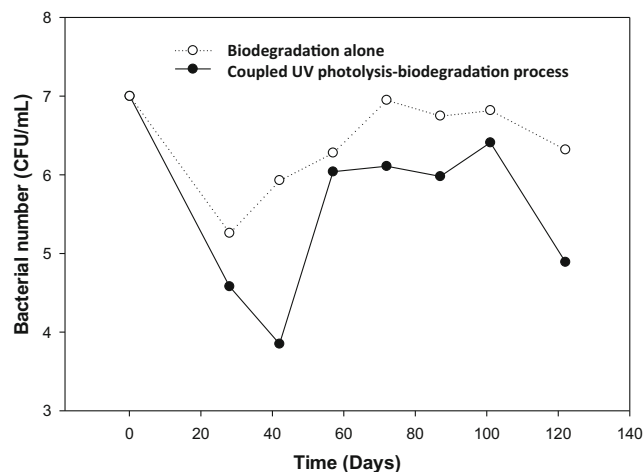
### Metabolite analysis

Degradation of BDE-209 by photolytic reactions and microbial biodegradation in the NBB involved cleavage of the aromatic ring, chemical/biological debromination and hydroxylation. The major groups of intermediates resulting from the coupled UV photolysis-biological process include (1) hydroxylated PBDEs (OH-PBDEs), e.g., 2-bromo-3, 5-dimethoxybenzyl alcohol; (2) debrominated PBDE congeners, e.g., BDE-99 and BDE-47; and (3) polybrominated

dibenzofurans (PDBFs) such as 2,8-dibromodibenzofuran and 4-bromo-dibenzofuran (Table S3), in addition to the bromide (Fig. 4). The concentration of bromide, generated from BDE-209, in the coupled process increased from  $23.50 \text{ mg L}^{-1}$  on day 84 to  $43.74 \text{ mg L}^{-1}$  on day 131 (total 48 days). Compared to the coupled process, the change in concentration (from day 84 to day 131) of bromide was higher with UV photolysis alone, increasing from  $23.06$  to  $53.53 \text{ mg L}^{-1}$ , and lower under biodegradation alone, increasing from  $22.96$  to only  $29.90 \text{ mg L}^{-1}$ . These results suggest that both chemical and biological debromination of BDE-209 occur during the coupled NBB process. The rate of bromide generation, derived using a two-variables liner equation from day 84 to day 131, from BDE-209 degradation in the NBB decreased in the order UV photolysis alone ( $0.63 \text{ mg L}^{-1} \text{ day}^{-1}$ ) > coupled UV photolysis and biodegradation ( $0.42 \text{ mg L}^{-1} \text{ day}^{-1}$ ) > biodegradation alone ( $0.14 \text{ mg L}^{-1} \text{ day}^{-1}$ ).

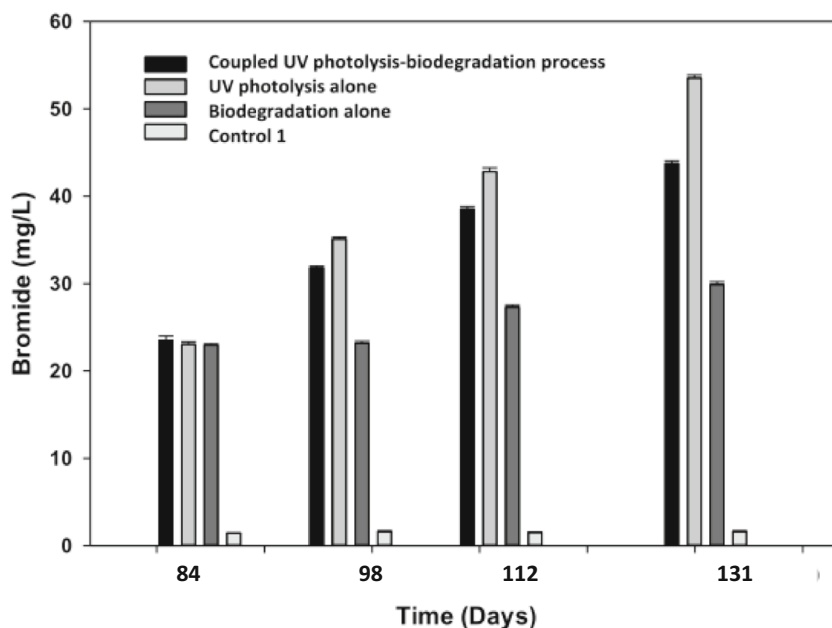
### Identification of various bacterial genera present in the NBB

Bacterial strains isolated and their ability to utilize PBDE congeners during the coupled UV photolysis-biodegradation process are summarized in Table 2. Thirty-seven single colonies grew on agar plates containing BDE-209 as the sole carbon source, indicating that these strains had the ability to biodegrade PBDEs. The bacterial isolates include (1) *Pseudomonas* spp.: Strain No. UV-D, UV-H, UV-I and UV-J; (2) *Microbacterium* spp.: Strain No. UV-A, UV-C and UV-E; (3) *Stenotrophomonas* spp.: Strain No. UV-F and UV-G; and (4) *Paracoccus yeei*: Strain No. UV-B. Of these, six bacterial strains, consisting of four *Pseudomonas* spp. (Strain No. UV-D, UV-I, UV-J and UV-K) and two *Microbacterium* spp. (Strain No. UV-A and UV-C), were found to have “very strong” abilities to utilize BDE-209 as a sole carbon source



**Fig. 3** The number of bacteria present during BDE-209 degradation by coupled UV photolysis-biodegradation in the NBB as measured by bacterial counts on BDE-209 agar plates. Y axis is a log scale

**Fig. 4** Bromide concentration released in the NBB by coupled UV photolysis-biodegradation process used for the treatment of BDE-209



(Table 2). One *Pseudomonas* sp. (Strain No. UV-J) had a strong ability to utilize BDE-15 as sole carbon source. Two *Pseudomonas* spp. (Strain No. UV-J and UV-K) were able to utilize strongly a defined mixture of 38 PBDE congeners as carbon sources. Figure S1 shows the phylogenetic trees of the identified bacteria based on their 16S rRNA gene sequences. Four bacterial species were identified, i.e., *Paracoccus yeei*, *Pseudomonas geniculate*, *Microbacterium laevaniformans*, and *Pseudomonas stutzeri* which are known to be widely distributed in PBDE/ECs-contaminated soil (Chou et al. 2013; Sowada et al. 2014; Wu et al. 2018).

### The functional genes associated with BDE-209 biodegradation present in the isolated bacterial strains

The results for the PCR-based detection of specific functional genes related to BDE-209 biodegradation in total genomic DNA extracts from the NBB are shown in Fig. S2. Table 2 shows RNA expression of the specific functional genes related to BDE-209 biodegradation in the various isolated bacterial strains. There were positive signals for three dioxygenases (C23O, ARHD and Rf) and two dehalogenases (RdhA and Rdases) in eight bacterial strains isolated from the coupled UV photolysis-biodegradation process. There was no signal present for the negative control (TCEse and PceA primers) in any of the bacterial strains tested. The UV-F strain showed the most positive responses, having five functional genes (C23O, ARHD, Rf, RdhA and Rdase) present. The UV-A and UV-K strains gave positive signals for three functional genes (C23O, ARHD, and RdhA). No signals were detected for any of the selected genes in three of the isolated strains (Strain No. UV-B, UV-C, and UV-E). Signals also were present for dioxygenases. These were C23O (eight strains: UV-A,

UV-D, UV-F, UV-G, UV-H, UV-I, UV-J, UV-K), ARHD (three strains: UV-A, UV-F, and UV-K), and Rf (three strains: UV-D, UV-F, and UV-G). Five *Pseudomonas* spp. (Strain No. UV-D, UV-H, UV-I, UV-J and UV-K), two *Stenotrophomonas* spp. (Strain No. UV-F and UV-G), and one *Microbacterium* sp. (Strain No. UV-A) were shown to express the C23O gene. Positive signals for the dehalogenases RdhA and Rdase were found for six bacterial strains. Specifically, six bacterial strains (UV-A, UV-F, UV-H, UV-I, UV-J, and UV-K) were positive for RdhA in their genomic DNA, while only one strain (UV-F) was positive for Rdase.

### Discussion

#### Advantages of the NBB coupled UV photolysis-biodegradation process

The coupling of UV-photolysis with biodegradation treatment in a NBB system has been shown here to be more effective at removing BDE-209 compared to biodegradation alone. There are several practical design advantages to the NBB for future use in soil remediation. First, UV photolysis commences instantly and at a uniform level once the LED UV lights are activated, and the intensity of exposure is easily measured at the surface of the inner quartz tank. The use of LED UV lamps reduces the amount of heat generated and saves significant amounts of energy compared to conventional UV light (Chang et al. 2020). The longer life cycle of the LED-UV lamps and lower energy demands for cooling to maintain temperature at a level suitable for bacterial growth are expected to reduce energy inputs. In addition, the UVA used to induce photolysis in the NBB does not induce damage to the bacterial

**Table 2** Bacterial strains identified as present in the NBB, and the presence of functional genes related to BDE-209 biodegradation during coupled UV photolysis-biodegradation process

Strain No.	Bacterial strains (NCBI Similarity Identification)	Utilization of PBDEs as carbon source			Response to target primers with functional genes related to BDE-209 biodegradation					
		BDE-209	BDE-15	Mixed 38 PBDEs	C230	ARHD	RF	RdhA	RDase	TCEse PceA (Negative control)
UV-A	<i>Microbacterium</i> sp. (97%)	4	1	1	+	+	-	+	-	-
UV-B	<i>Paracoccus yeei</i> (97%)	2	1	1	-	-	-	-	-	-
UV-C	<i>Microbacterium</i> sp. (97%)	4	0	0	-	-	-	-	-	-
UV-D	<i>Pseudomonas geniculata</i> (98%)	4	0	0	+	-	+	-	-	-
UV-E	<i>Microbacterium laevaniformans</i> (97%)	3	1	1	-	-	-	-	-	-
UV-F	<i>Stenotrophomonas</i> sp. (98%)	3	1	1	+	+	+	+	+	-
UV-G	<i>Stenotrophomonas</i> sp. (97%)	3	1	1	+	-	+	-	-	-
UV-H	<i>Pseudomonas stutzeri</i> (96%)	2	2	0	+	-	-	+	-	-
UV-I	<i>Pseudomonas</i> sp. (97%)	4	0	0	+	-	-	+	-	-
UV-J	<i>Pseudomonas</i> sp. (97%)	4	3	4	+	-	-	+	-	-
UV-K	<i>Pseudomonas stutzeri</i> (97%)	4	1	4	+	+	-	+	-	-

0: No reaction
1: Weak reaction
2: Medium reaction
3: Strong reaction
4: Very strong reaction

DNA to a significant degree because it is not absorbed by native DNA. The acclimated UV-resistant bacteria in the NBB are highly capable of BDE-209 utilization; they grow well in the NBB, biodegrading BDE-209, PBDEs and OH-PBDEs. Furthermore, the heat generated by LED-UV irradiation helps maintain a steady temperature (on average 31 °C) in the NBB thereby enhancing the rate and extent of BDE-209 biodegradation in the clay/water system evaluated. The first-order-rate constant (*k*) for BDE-209 biodegradation compared by coupled UV photolysis and biodegradation in the NBB was  $1.31 \times 10^{-2} \text{ day}^{-1}$ , which is greater than that observed for a batch experiment where dark-brown glass serum bottles were used and biodegradation was combined with UV irradiation at room temperature ( $k = 1.29 \times 10^{-2} \text{ day}^{-1}$ ) (Chou et al. 2013).

Sequential UVB photolysis-biodegradation process was shown previously effectively remove BDE-209 using a clay/water system (Chang et al. 2020). Overall, the sequential process is a more effective design than the coupled UV photolysis-biodegradation process (described herein) or biodegradation alone when the processes are evaluated using their rates of BDE-209 degradation. The first-order rate constants (*k*) are as follows: sequential UVB photolysis-biodegradation process ( $k = 0.026 \text{ day}^{-1}$ ) > coupled LED-UVA photolysis-biodegradation process ( $k = 0.013 \text{ day}^{-1}$ ) > biodegradation alone ( $k = 0.010 \text{ day}^{-1}$ ). The UVB irradiation treatment with high energy intensity was intended to generate more free radicals that cleave C-Br bonds of BDE-209 present in the clay/water slurry system (Eriksson et al. 2004). The molar absorptivity of PBDEs in the

UVB region is much greater than that in the UVA region (Pan et al. 2016). Therefore, the higher rate constant for BDE-209 degradation in the clay/water system by photolysis with UVB irradiation ( $0.017 \text{ day}^{-1}$ ) as compared to photolysis by UVA irradiation ( $0.011 \text{ day}^{-1}$ ) could plausibly indicate greater formation of OH-PBDE and PBDE intermediates in the former case (UVB photolysis) which would be more susceptible to biodegradation including use as a carbon source and/or as an alternative carbon source via co-metabolism. Pretreatment of high-molecule-weight aromatic PAH mixtures, containing anthracene, pyrene, benz[a]anthracene, and dibenz[a,h]anthracene, with UV irradiation was shown to accelerate biodegradation by an enriched culture of a *Sphingomonas* sp. and *S. yanoikuyae* (Lehto et al. 2003). Potential disadvantages of the sequential UVB photolysis-biodegradation process compared to the coupled UVA photolysis-biodegradation process are the need for extra time to transfer the clay/water system from the first-stage UVB photolysis system into the second-stage system for biodegradation, and secondly the amount of energy required for UVB irradiation is greater than that required for continuous LED UVA irradiation. Furthermore, the coupled LED UVA photolysis-biodegradation process used here to successfully treat BDE-209 is an energy and cost-saving approach. The NBB is designed to have cooling fins and is able to recycle most of waste energy produced during the continuous UVA irradiation as heat loss and thus the system is able to maintain the steady temperature required for bacterial degradation. The UV-resistant bacteria present in the reactor are therefore able to accelerate the removal of BDE-209 and its intermediates by photolysis/biodegradation in parallel within the NBB. The advantages of this system mean that the NBB should be able to decompose other POPs/ECs and therefore will be very useful for the remediation of a wide range of contaminated environments and ecological system.

**Bacterial strains isolated from the NBB system are involved in PBDEs biodegradation**

Bacterial strains isolated from the NBB operating with coupled BDE-209-biodegradation have the established ability to utilize PBDEs and/or a variety of other aromatic compounds. For example, *Pseudomonas aeruginosa* has been shown to be involved in the aerobic debromination of BDE-209. Evaluation of biodegradation intermediates showed that *P. aeruginosa* degraded BDE-209 into lower brominated PBDEs and OH-PBDEs through debromination and hydroxylation of the aromatic rings (Liu et al. 2019). Furthermore, *P. stutzeri* has been shown to utilize BDE-47 as a sole carbon source and achieved almost complete (97.94%) biodegradation during a two-week incubation (Zhang et al. 2013b). *Mycobacterium* spp., which are distributed widely in soils, have the ability to biodegrade aromatic pesticides and contaminants, such as the acetanilide herbicides (e.g., propanil) and



benzo(a)pyrene (Hou et al. 2015; Qin et al. 2017). *Microbacterium* sp. have been shown to biodegrade BDE-47 as sole carbon source under aerobic condition (Zhang et al. 2013a). A *Stenotrophomonas* strain isolated from soils at a PBDEs-contaminated e-waste recycling site was able to effectively degrade BDE-209 (55.15% of 65  $\mu\text{g l}^{-1}$ ) under aerobic conditions during a 30-day incubation (Wu et al. 2018), and *Paracoccus yeei* has been shown to utilize benzo[a]pyrene as a sole carbon and energy source (Sowada et al. 2014).

UVA irradiation can adversely affect microbial activity and hence inhibit biochemical reactions in the NBB. Figure 3 shows that the number of bacteria present during the coupled UV photolysis-biodegradation process is less than for biodegradation alone. UVA light has been reported to generate singlet oxygen ( $^1\text{O}_2$ ) that can damage bacterial DNA via indirect photosensitization reactions (Rastogi et al. 2010). However, the microbial community within the BDE-209-clay/water system has been reported to have a high tolerance for continuous UVA irradiation, as well as the ability to bring about PBDEs biodegradation (Chang et al. 2019). *Pseudomonas* spp., *Microbacterium* spp., and *Stenotrophomonas* spp. have all been identified as being involved in BDE-209 biodegradation under continuous UVA irradiation in the NBB, and these are known to be radiation resistant bacteria. Specifically, they are usually able to express enzymes capable of repairing the damaged DNA created by irradiation and thus are able to survive high levels of radiation. Specific chemicals, such as ectoine from *Stenotrophomonas* spp., have been shown to overcome radiation-mediated oxidative damage in an extreme UV irradiation environment (Sajjad et al. 2018).

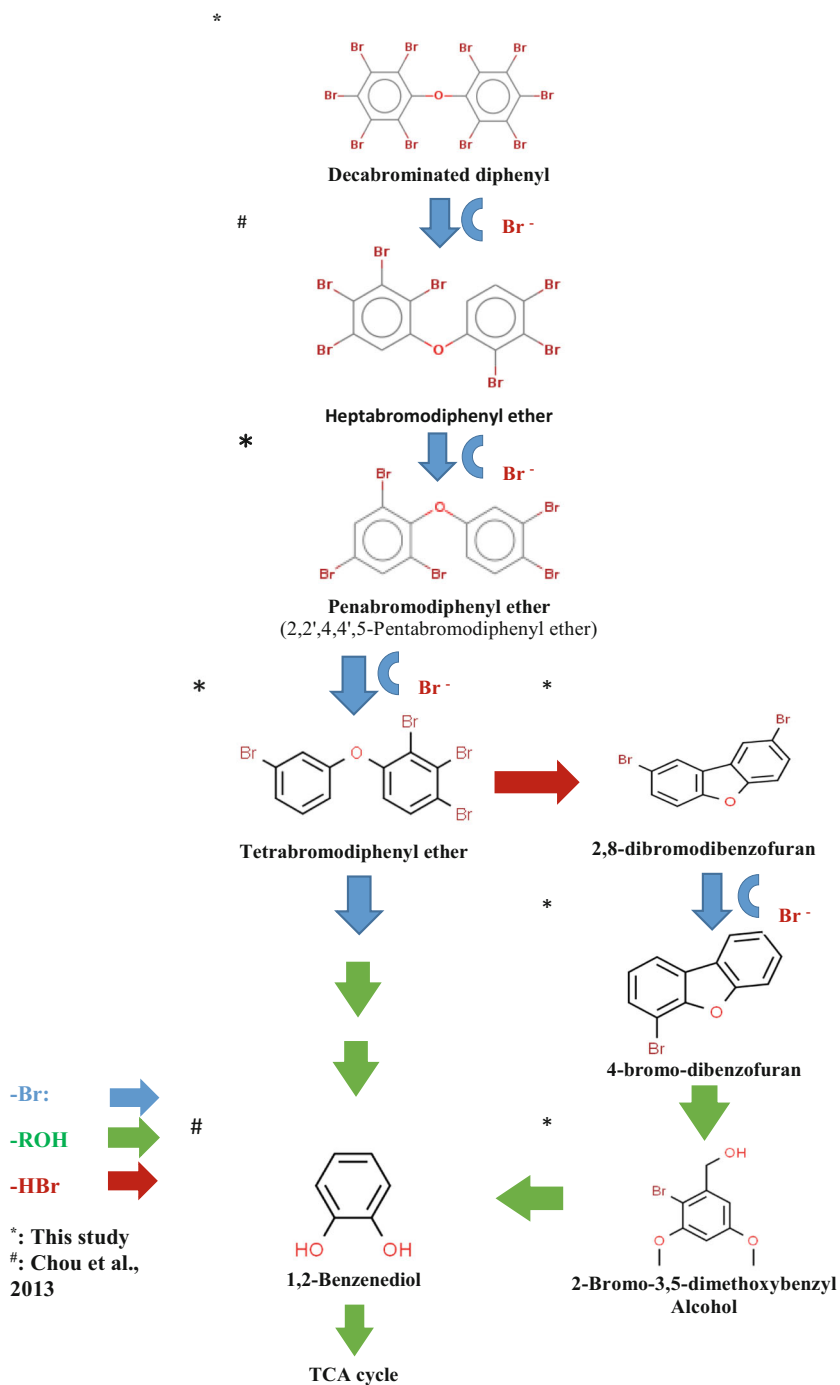
### The mechanisms of BDE-209 degradation present in the NBB system

A possible pathway for BDE-209 degradation by coupled UV photolysis and biodegradation is shown in Fig. 5. This is based on several lines of evidences: (1) the intermediates detected during our study; (2) the release of bromide from BDE-209, and (3) presence of genes encoding functional enzymes related to BDE-209 biodegradation. Various degradation intermediates, such as PBDE congeners, OH-PBDEs, PBDFs, and bromide, are generated by a complex series of photolytic and biological reactions within the clay-water slurries of the NBB system. Consecutive reductive debromination with intramolecular elimination of HBr is a predominant pathway associated with solid phase PBDEs photolysis (Pan et al. 2016). In the present study, spontaneous debromination is known to occur during UV photolysis of intermediates following ring cleavage of PBDEs. Higher brominated PBDEs, e.g., BDE-209, can absorb long wavelength 365 nm-UVA light and undergo consecutive debrominated to give lower brominated PBDEs, which are relatively more soluble in clay-water slurries. The accumulation of lower brominated BDE-99 and

BDE-47 in the NBB also occurs during the photolytic debromination of BDE-209 in soils (Söderström et al. 2004). Moreover, the formation of PBDFs can occur via the intramolecular elimination of HBr from either lower brominated PBDEs or OH-PBDEs which are also formed by homolytic C-O bond cleavage of lower brominated PBDEs. However, it should be noted that PBDF derivatives have disappeared by the end of the present experiment (day 120), which supports the notion that there are multiple reactions and coupled processes involving UV photolysis and aerobic biodegradation occurring within the reactors and that bring about the removal of PBDFs.

Aerobic biodegradation of lower brominated PBDEs and OH-PBDEs has been shown to involve several mechanisms. First, cleavage of the aromatic rings of lower brominated PBDEs occurs via a series of reactions involving hydroxylation. Alternatively, the cleavage of benzene rings might occur in various different bacterial species under aerobic conditions, based on the presence of positive signals for genes encoding various dioxygenases. Thus, bromine substituents of the lower brominated PBDEs could be substituted for by an OH group (via hydroxylation) thus generating hydroxylated biometabolites. Genes encoding a number of dioxygenases, such as C23O, ARHD, and Rf, are known to be involved in the biodegradation of aromatic hydrocarbons by the various bacterial species that have been identified in this study (Table 1). These dioxygenases employ different approaches when utilizing BDE-209 and its degradation intermediates. Positive PCR reactions for these dioxygenases during coupled UV photolysis/biodegradation suggest that these different enzymes are involved in the aerobic biodegradation of BDE-209, including the oxidative cleavage of PBDEs and other metabolites such as catechol. This hypothesis is supported, firstly, by the fact that a wide variety of C23O genes are present in the NBB bacterial species identified with the reactor. These C23O enzymes are known to bring about meta-cleavage at specific unbrominated ring positions, and have also been identified as present during BDE-209 biodegradation in a soil slurry microcosm (Chou et al. 2016). An increase in expression of between 30-fold and 3,000-fold has been found for biphenyl and ethylbenzene dioxygenases during the biotransformation of mono-BDE through to penta-BDE congeners (Robrock et al. 2011). Moreover, the ARHD genes are able to degrade polychlorinated biphenyls (PCB), which have a chemical structure similar to the PBDE congeners detected in the present study. These enzymes consist of ring hydroxylation dioxygenases that have cysteine and histidine residues coordinated with a 2Fe-2S cluster, as well as hydroxylation dioxygenases (Kitagawa et al. 2001). Rf genes encode the larger  $\alpha$ -subunit of a terminal dioxygenase. This gene has been used as an oxygenase indicator when there is aromatic substrate specificity and when dioxygenase activity is the rate-limiting step. The  $\alpha$ -subunit amplified here is the catalytic component

**Fig. 5** Proposed pathways for coupled UV irradiation-biodegradation process used for BDE-209 treatment in the NBB



and contains two conserved regions, namely the [Fe<sub>2</sub>-S<sub>2</sub>] Riseker center and the mononuclear iron binding domain. These two centers have been shown to be involved in consecutive electron transfer to dioxygen molecules (Yu et al. 2019a).

In addition, it is plausible that biological reductive debromination is occurring in the reactor because there are positive signals for genes encoding dehalogenases, and this enzymatic reaction is known to generate significant amounts of bromide. Dehalogenation is the key step in the biodegradation of halogenated aromatics. Various dehalogenases, such as

RdhA and Rdase, have been shown to be involved in the aerobic dehalogenation of aromatic organohalide compounds (Arora and Bae 2014). Three bacterial species identified in the present study, *Pseudomonas* spp. (UV-H, UV-I, UV-J and UV-J), *Stenotrophomonas* sp. (UV-F) and *Microbacterium* sp. (UV-A), are known to be involved in the biodegradation of halogenated aromatic hydrocarbons. One enzyme responsible for catalyzing organohalide respiration under aerobic conditions is reductive dehalogenase (Rdase). Rdase c of a cobalamin centered catalytic subunit A (RdhA) and a

membrane anchoring subunit B. Genes encoding Rdase and RdhA are likely to be involved in the reductive debromination of BDE-209 by the various aerobic/facultative anaerobic bacterial species identified in this study. RdhA proteins have several conserved domains, including two iron-sulfur [4Fe-4S] cluster-binding motifs and a twin-arginine signal motif that is used for translocation to or translocation across the cell membrane (Hug and Edwards 2013). One aerobic Gram negative bacteria, *Comamonas* sp. 7D-2, has been shown to be able to completely degrade the brominated aromatic herbicide bromoxynil, and in the process it releases two equivalents of bromide; this was carried out under aerobic conditions and a specific Rdase was identified. This enzyme has key features in common with the anaerobic respiratory RdhAs, such as a complex of a RdhA domain and a NAD(P)H-dependent oxidoreductase domain (Chen et al. 2013). Finally, a microbial consortium, including three of our identified species, i.e., *Microbacterium* sp., *Stenotrophomonas* sp., and *Pseudomonas* sp., has been shown previously to encode RdhA genes capable of aerobic BDE-209 biodegradation (Yu et al. 2019b).

In the model described above, BDE-209 degradation occurs by UV irradiation and biodegradation during the coupled UVA photolysis-biodegradation process. The rate of bromide generation by photolysis alone ( $0.63 \text{ mg L}^{-1} \text{ day}^{-1}$ ) is faster than by biodegradation alone ( $0.14 \text{ mg L}^{-1} \text{ day}^{-1}$ ) indicating that the process of chemical debromination is faster than the biological debromination. Thus, UV photolysis in the NBB might include the major process by which PBDEs, especially BDE-209, are initially decomposed. Specifically, chemical debromination will plausibly break the Br-C bonds of BDE-209 effectively when significant amounts of free radicals are being created by UV irradiation resulting in less BDE-209 being biodegraded by the Da-An bacterial community. A previous study measured the rates of breakdown of a mixture of fifteen PBDEs by photolysis. When the rates for higher brominated PBDEs were compared to those for lower brominated PBDEs (Eriksson et al. 2004), the rate of BDE-209 degradation ( $4 \times 10^6, \text{ s}^{-1}$ ) was a 100 fold higher than that of BDE-155 degradation ( $4.1 \times 10^4, \text{ s}^{-1}$ ) and was 570 fold higher than that of BDE-47 ( $7.0 \times 10^3, \text{ s}^{-1}$ ).

## Conclusion

This study developed a coupled LED UVA photolysis-biodegradation process in a NBB and in the process successfully maximized the degradation rate of the BDE-209 present in a clay-water slurry, compared to either UV photolysis alone or biodegradation alone. The NBB was designed so that the multiple chemical/biological reactions brought about by UV photolysis and biodegradation occur together in synergy. The major derivatives created by this system during the

decomposition of BDE-209 include OH-PBDEs, lower brominated PBDE congeners, PBDFs, and significant amounts of bromide. The prediction of a possible pathway for BDE-209 degradation via coupled photolysis-biodegradation was developed, based on the above-mentioned intermediates. Specific UV-resistant bacterial strains, e.g., *Stenotrophomonas* sp., *Pseudomonas* sp., and *Microbacterium* sp., were identified during the coupled UV-biodegradation process and these strains potentially have the ability to utilize BDE-209 as a sole carbon source. Since the chemical structure of PBDEs consists of two aromatic benzene rings with different numbers of bromides, this structure is vulnerable to sequential benzene ring-cleavage reactions by various dioxygenases. The presence of ring-cleavage oxygenases such as C23O and dehalogenases such as RdhA in this aerobic system opens up possibilities for basic research into these enzymes. Furthermore, these enzymes have a wide potential applications as part of an integrated remediation system for PBDEs. The results presented here provide the basis for an effective green remediation technology for the treatment of POPs/ECs when they are associated with soil geosorbents. Our findings provide a good starting point for the development of an ex situ integrated remediation system that couples UV photolysis with biodegradation and that is able to degrade BDE-209 effectively. Future studies using whole soils and other recalcitrant POPs/ECs are needed to broaden the application of this promising technology. In addition during such analyses, changes in expression levels of the various functional genes associated with and related to BDE-209 biodegradation need to be quantified by qPCR.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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