DECOMPOSITION OF BDES BY CHEMICAL AND BIOLOGICAL TREATMENTS

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Abstract
Treatment of highly halogenated pollutants from the environment is quite hard due to their highly extreme persistency, recalcitrance and bio-unavailability. In present study, brominated diphenyl ethers (BDEs) were selected for the hybrid treatment by the combination of two kinds of treatment methods; a reductive debromination by nano scaled zero valent iron (nZVI) and a biological oxidation by *Sphingomonas* sp. PH-07. Debromination of deca-BDE by nZVI resulted in debrominated products such as nona- to mono-BDEs after 15 days reaction. Following the anaerobic debromination process, reaction mixture was aerobically treated with *Sphingomonas* sp. PH-07 strain for additional 4 days. During bacterial treatment, low brominated diphenyl ethers were further biologically transformed to bromophenols and other prospective metabolites in our experiments. This combination methodology could also give an insight to develop a remediation strategy of highly halogenated environmental pollutants in contaminated sites.

Introduction
Polybrominated diphenyl ethers (PBDEs) are one of the representative halogenated chemicals in the environments due to its massive industrial uses for flame retardant additives in the production of electronic appliances, polyurethanes, plastics and textiles. PBDEs are consisting of 209 congeners by numbers and positions of bromine atoms on the diphenyl ether and major components are penta-BDE, octa-BDE, and fully brominated deca-BDE in the industrial products. Since the active uses of these flame retardants, various concentrations of PBDEs have been reported in many environmental matrices including water, soil, sediment, and biota sample[1].
Zero valent iron particles effectively dehalogenate many kinds of halogenated aliphatic and aromatic hydrocarbons under anaerobic condition[2-4]. Many investigations of ZVI reduction were highly focused on permeable reactive barriers for the remediation of groundwater which were contaminated by aliphatic chlorinated solvents such as trichloroethylene[3,5-6]. Recently the application size of ZVI expanded from micro size granules to nano scale particles (<100 nm) due to their large surface area and dependent high reactivity. In addition, nano sized particles are easier to be suspended in water phase and this physical property can play an major role in transport and diffusion process at the remediation application[7]. However, the reductive dehalogenation itself is still not be an impeccable method for the removal of halogenated pollutants, which can produce more toxic substances as intermediate or have low reactivity to low halogenated compounds.
Aerobic microbial oxidation could be a faster alternative and this process could mineralize all organic structures to simple elements such as carbon dioxide and others. However their involved enzyme systems concerned to the degradation are usually inhibited by the structural interferences of derivatives, especially for halogenated pollutants. In the aerobic degradation and transformation of PBDEs, a few studies were carried out and low brominated congeners were assessed by the aerobic enzymatic reactions[7-9]. The number and position of bromine atoms are critical for the transformation and even biotransformation of 2,4,4'-triBDE was available, but 2,4,6-triBDE was not accessed[7]. In this study, we demonstrated a sequential treatment of reductive debromination using nZVI and aerobic biodegradation and biotransformation for the first to end decomposition of deca-brominated diphenyl ether (Fig. 1).

Materials and Methods
Chemicals. Diphenyl ether and decabromodiphenyl ether were purchased from Sigma-Aldrich. Dimethylsulfoxide (DMSO), acetone, tetrahydrofuran, toluene, and nutrient agar were purchased from Merck (Darmstadt, Germany). $Fe_2(SO_4)_3$ and $NaBH_4$ were used to synthesize nano-size ZVI. Minimal salt medium (MSM) was prepared by the method described by Fortnagel et al[10]. All other chemicals and solvents used were of the highest purity available. All water phase solutions concerned with nZVI were prepared using distilled and deionized water which was already deoxygenated by purging of argon gas for 1 h.

Synthesis of nZVI and preparation of bacterial cell. The total amount of synthesized nZVI was controlled by the amount $Fe_2(SO_4)$ in each reaction. 2.1 mM of $Fe_2(SO_4)_3$ was dissolved in 100 mL water and 4.2 mM of $NaBH_4$ solution was added slowly to reduce Fe^{2+} to Fe^0 in 250 mL beaker. Synthesized nZVI was washed three times with degassed pure water to remove remained $NaBH_4$, then nZVI solution was sonicated for 10 min to inhibit the self aggregation of nano particle. Finally this nZVI was rapidly transferred to 15 mL test tube under the argon atmosphere to prevent the oxidation of nZVI and kept for the next application. Physical properties were already characterized by previous paper following this synthesizing method by Kim et al[4].

The PH-07 strain (NCBI Genbank DQ185574) was continuously maintained in our laboratory. For the preparation of resting cells, this strain was inoculated in 400 mL of MSM (2 L Erlenmeyer flask) with 1 g/L of diphenyl ether as a sole carbon source. This flask was incubated for 4 days in a darkened shaking incubator (160 rpm, 28°C) and the whole culture suspension was harvested by centrifugation (10,000 × g, 20 min, 4°C). The obtained pellet was washed three times with sterile 20 mM phosphate buffer (pH 7.3) and the optical density of the final cell suspension was adjusted to 4.0 at 578 nm in distilled water and MSM.

Debromination of deca-BDE. Deca-BDE was prepared by dissolving in tetrahydrofuran (1 g/L) and then 100 µL of this stock solution was spread on the freshly prepared nZVI in test tube. After gentle vaporization of solvent by agron gas fuzzing, 6 mL of degassed water was poured and argon gas was injected to remove oxygen in the headspace of test tube before sealing the Teflon-coated test tube cap. These test tubes were stored horizontally in the darkened shaking incubator. At the each sampling points (0, 5 d, 10 d, 15 d, 20 d), triplicate samples were taken. Before the extraction, a strong magnet was applied to retain the iron particle in the bottom of test tube. Water phase was transferred to another test tube and extracted with 4 mL (2 × 2 mL) toluene, and analytes existing at iron particle phase were extracted with 5 mL of acetone:toluene (1:1) with sonication. After removing of water by sodium sulfate, two extracts were combined and final volume was adjusted to 1 mL through solvent vaporization by nitrogen gas purging.

Sequential treatment of bacterial cell to the mixtures of debrominated diphenyl ethers. After the debromination process, all screw caps of test tubes were opened and 6 mL of prepared resting cells of PH-07 strain in MSM and DW was separately poured to each test tube. All test tubes were further incubated additional 4 days at the same condition while the screw caps were not closed tightly to supply fresh air which contains oxygen. As controls, debrominated test tubes which were not added resting cells were also incubated at same condition. After the 4 days incubation, all these reacted test tubes were taken and extracted as same method describe above. After the quantification of remained BDEs in all samples, they were additionally reacted with BSTFA (20 µL in 1 mL) to form trimethylsillyl (TMS) derivatives of biologically produced intermediates such as bromophenols and hydroxylated BDEs. Major intermediates which were transformed to TMS forms were analyzed belong to the further instrumental analysis and quantified when authentic standards were available in this study.

Instrumental Analysis. A gas chromatograph with electron capture detector (GC-ECD; Agilent 6890, Agilent Technology) and Polaris Q Ion Trap gas chromatograph and mass spectrometer (GC-MS) were employed for the qualitative and quantitative analysis of decaBDE and debrominated diphenyl ethers. DB5-MS (30 m × 0.25 mm × 0.25 µm) and HT5-MS (15m × 0.25 mm × 0.25 µm) columns were used in this study which were obtained from Agilent Technology Inc. Splitless injection mode was selected and 1µL of samples were injected. The oven temperature was held at 120°C for 5 min and increased at 5°C /min rate to 320°C and finally held for 5 min. The ion source temperature was 270°C and ionization energy was 70 eV through whole mass analysis.

Results and Discussion

Debromination of BDEs using nZVI. In the anaerobic process, 1 mg of deca-BDE was gradually debrominated in test tube and about 67% of deca-BDE was transformed to the lower BDEs through the nZVI treatment for 20 days (Fig. 2). GC-MS analysis revealed that the most abundant debrominated homologue was hexa-BDEs throughout the incubation. The formation of tri-BDEs which is a biologically accessible homologue, was our major interest through the debromination step which can be connected to further biological transformation. After 20 days incubation of deca-BDE with nZVI, tri-BDEs were significantly produced and their molar percent was about 6.4 mol among the whole quantified congeners including deca-BDE. Among the detected tri-BDEs, 2,4,4'-triBDE was the most dominant congener which were originated from deca-BDE. Since the objective of this study was to evaluate the sequential treatment with anaerobic nZVI reaction and successive aerobic bacterial transformation rather than the computation of debromination kinetics which were already reported[11] we focused

on the appearance of tri-BDEs which are more accessible to aerobic biotransformation. In spite of the appearance of tri-BDEs from the debromination decaBDE with nZVI, another approach was needed to understand the sequential treatment of anaerobic and aerobic reactions more clearly because, the tri-BDEs were not sufficiently produced from the debromination process.

Biological treatment of debrominated BDEs. After the debromination reaction, the debrominated sets of deca-BDE were subsequently reacted with a diphenyl ether degrading bacterium *Sphingomonas* sp. PH-07 for further biological oxidation under aerobic condition. From the successive biological treatment, not only BDEs but also ether bond cleaved products and hydroxylated BDEs were fairly detected, however, it was tricky to show the decrease of all target compounds such as tri- and di-BDEs. The detected amounts and difference compare with controls were so chaotic to interpret data because debrominates especially for range of tri-BDEs to mono-BDEs were poorly existed already in the debromination process. However, the existence of dibromophenols and monobromophenols were detected in PH-07 strain treated reaction tubes and they were confirmed with authentic compounds at same m/z values and retention times. As the intermediates, mono bromophenols and dibromophenols were detected from all sets of biological treatments. Samples of biological reaction amended with MSM showed high level of brominated intermediates than in DW amended samples. But, the addition of diphenyl ether to each test tube as supplementary carbon substrate, reduced the formation of bromophenols, but only phenol was mainly produced as a major intermediate from the biodegradation of diphenyl ether by *Sphingomonas* sp. PH-07.

In conclusion, we confirmed the possibilities of successive treatment of decaBDE using nZVI and biological oxidation, and in further we could optimize for complete decomposition of deca-BDE through this nano-bio coupling treatment. This treatment method also could be applied for highly halogenated persistent organic pollutants such as octachlorinated dibenzo-*p*-dioxins, dibenzofurans and decachlorinated biphenyls.

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Fig. 1. The scheme of hybrid treatment of deca-BDE. The combination of reduction and oxidation could enhance the environmental remediation processes.

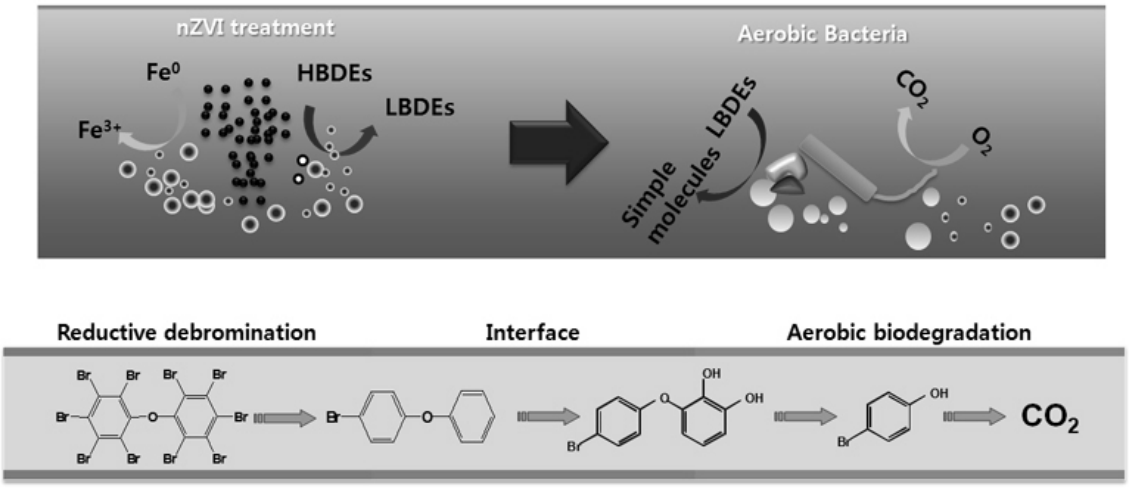

Fig. 2. GC/MS chromatograms of deca-BDE treated with nZVI. Peaks of debrominated BDEs were grouped into tri- to nona-BDEs.