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4 **Nano/bio treatment of polychlorinated biphenyls with evaluation of**
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6
7 **comparative toxicity**
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10 Thao Thanh Le^{a#}, Hoang Khanh Nguyen^{a#}, Jong-Rok Jeon^b, Arokiasamy J. Francis^c, Yoon-Seok
11 Chang^{a*}
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16
17 ^aSchool of Environmental Science and Engineering, POSTECH, Pohang 790-784, Republic of
18 Korea
19

20
21 ^bCorporate R&D, LG Chem Research Park, Daejeon 305-380, Republic of Korea
22

23
24 ^cDivision of Advanced Nuclear Engineering, POSTECH, Pohang 790-784, Republic of Korea;
25 and Environmental Sciences Department, Brookhaven National Laboratory, Upton, New York,
26 USA
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38 *# Authors have the same distribution*
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43
44 *** Corresponding author**
45

46 **Prof. Dr. Yoon-Seok Chang**
47

48 School of Environmental Science and Engineering,
49

50 Pohang University of Science and Technology (POSTECH),
51

52 Pohang 790-784, Republic of Korea
53

54 Tel.: +82 54 279 2281; Fax: +82 54 279 8299
55

56 E-mail address: yschang@postech.ac.kr (Y.-S. Chang)
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Abstract

The persistence of polychlorinated biphenyl (PCB) Aroclor 1248 in soils and sediments is a major concern because of its toxicity and presence at high concentrations. In this study, we developed an integrated remediation system for PCBs using chemical catalysis and biodegradation. The dechlorination of Aroclor 1248 was achieved by treatment with bimetallic nanoparticles Pd/nFe under anoxic conditions. Among the 32 PCB congeners of Aroclor 1248 examined, our process dechlorinated 99%, 92%, 84%, and 28% of tri-, tetra-, penta-, and hexachlorinated biphenyls, respectively. The resulting biphenyl was biodegraded rapidly by *Burkholderia xenovorans* LB400. Benzoic acid was detected as an intermediate during the biodegradation process. The toxicity of the residual PCBs after nano-bio treatment was evaluated in terms of toxic equivalent values which decreased from $33.8 \times 10^{-5} \mu\text{g g}^{-1}$ to $9.5 \times 10^{-5} \mu\text{g g}^{-1}$. The residual PCBs also had low cytotoxicity toward *Escherichia coli* as demonstrated by lower reactive oxygen species levels, lower glutathione peroxidase activity, and a reduced number of dead bacteria.

Keywords: Aroclor 1248, bimetallic nanoparticles Pd/nFe, dechlorination, nano-bio treatment, nanotoxicity

1. Introduction

Aroclor 1248 is a mixture of polychlorinated biphenyls (PCBs) with an average chlorine content of 48%. In the past, PCBs were manufactured and used widely in many industries as heat transfer fluids, organic diluents, plasticizers, pesticide extenders, dust-reducing agents, flame

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4 retardants, and in carbonless copy papers [1, 2]. Subsequently, the production of Aroclors and
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6 other PCBs was banned, and they are considered persistent organic pollutants under the
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8 Stockholm Convention of 2004 because of the risk they pose to the environment [3], and to
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10 human health, including hepatotoxicity, cancer [4], embryotoxicity, reproductive effects [1,5] ,
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12 immunotoxicity [6], and endocrine disruption [1,7]. The toxicities of Aroclors in terms of toxic
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14 equivalent (TEQ) values have been determined by measuring the concentration of 12 dioxin-like
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16 PCBs at the sub-ppm level [8]. The TEQs of Aroclors 1248 and 1254 were reported as 11.7 and
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18 7.7 $\mu\text{g g}^{-1}$ [9], and 15 and 21 $\mu\text{g g}^{-1}$ [8], respectively, and variation in TEQ values was found to
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20 depend on the ratio of each congener in the sample.
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27 Previously, PCB removal has been reported using anaerobic dechlorination via microbial
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29 reduction. Many PCB dechlorinating bacterial cultures such as *Dehalococcoides* and
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31 *Dehalobium* species have been isolated from sediments [10, 11, 12]. Mixed cultures of bacteria
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33 in sediments have also been used for Aroclor remediation [13, 14]. Aroclor biodegradation is
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35 also possible, in which its toxicity can be reduced preferentially by microbial reductive
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37 dechlorination of para- and meta- chlorines [13, 14, 15]. Some aerobic microbes such as
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39 *Burkholderia xenovorans* LB400 [16] and *Rhodococcus sp.* strain RHA1 [17] can cleave low
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41 chlorinated PCB congeners to produce chlorinated benzoates and pentanoic acid. One limitation
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43 of microbial remediation is that only a few cultures can dechlorinate mixtures of PCBs or allow
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45 cometabolic degradation of other coexisting halogenated compounds, and the microbial
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47 remediation generally requires a long period for complete mineralization.
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54 Recently, new catalysts have been developed for the dechlorination of halogenated
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56 organic compounds, such as nanoscale zero-valent iron (nZVI), and bimetallic nanoparticles
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58 Pd/nFe and Ni/nFe [18, 19, 20, 21]. These reports also showed that dechlorination efficiency and
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4 reaction rate could be accelerated by doping the surface of iron particles with transition metals
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6 such as Ni, Cu, Pt, or Pd. The catalytic dechlorination of Pd/nFe is attributable to H₂ production
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8 via the iron corrosion reaction of the zero-valent metallic iron and the hydrodechlorination of
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10 PCBs which is promoted by the atomic H generated on the Pd surface [19, 20]. The rate of
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12 dechlorination is influenced strongly by environmental conditions and the reaction time. Other
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14 factors that affect the efficiency of dechlorination include particle size, Pd loading, and whether
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16 buffered or unbuffered reaction media are used.
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22 A combination of chemical and biological treatments for halogenated compounds has
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24 been developed and is referred to as the nano-bio hybrid strategy. The nano-bio redox system
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26 comprises two operational stages: anaerobic dechlorination using nanoparticles (NPs) and
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28 subsequent aerobic mineralization by bacteria or fungal enzymes [19]. In our previous studies,
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30 we have applied this concept successfully to the removal of several individual halogenated
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32 pollutants, including Triclosan [22], deca-brominated diphenyl ether, BDE-209 [23] and 2, 3, 7,
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34 8-TCDD [24]. In the present study, we investigated the level of anaerobic dechlorination of
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36 PCBs after treatment with bimetallic Pd/nFe NPs followed by biodegradation of the products by
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38 *Burkholderia xenovorans* LB400 which is able to degrade low chlorinated congeners such as 2,4-
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40 CB or 2,2'-CB [25].
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50 **2. Experimental**

51 *2.1. Chemicals*

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54 Aroclor 1248, iron (II) sulfate (FeSO₄·7H₂O), sodium borohydride (NaBH₄), palladium
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56 acetate (Pd(COOCH₃)₂) were purchased from Sigma–Aldrich, USA. Acetone, ethanol, hexane,
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4 dimethyl chloride, and methanol were purchased from Merck, Germany. All other chemical are
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6 of the highest purity of analytical grade. The DI water (18.2 MΩ cm) used in the experiments
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8 was purified by Millipore Direct Q3 purification system. All aqueous solutions containing
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10 nanoparticles were prepared using degassed ultrapure water by purging with nitrogen gas for 2h.
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18 2.2. *Microorganism and culture*

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21 The bacterium *Burkholderia xenovorans* LB400 was grown aerobically in minimal salt
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23 medium (MSM) [26] with 5mM biphenyl as sole carbon source in a shaker incubator set at 160
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25 rpm, and 30°C. For the preparation of bacterial cells, the culture was grown to the log phase and
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27 harvested by centrifugation at 10,000 rpm, for 20 min, at 4°C. The cell pellet was washed twice
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29 by PBS buffer pH 7.4 and resuspended to a final OD_{600nm} of 2.0.
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37 2.3. *Pd/nFe nanoparticles synthesis*

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40 Zero valent iron (nFe) was prepared by reducing FeSO₄·7H₂O (3.1 g) with NaBH₄ (1.6 g)
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42 in 100 mL of degassed ultrapure water. The bimetallic nanoparticles palladium/zero valent iron
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44 (Pd/nFe) was prepared according to Bokare et al. [19] with a slight modification. Zero valent
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46 iron (nFe) was prepared by reducing FeSO₄·7H₂O (3.1 g) with NaBH₄ (1.6 g) in 100 mL
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48 degassed ultrapure water. The nFe was washed several times by ethanol and added to solution of
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50 Pd(COOCH₃)₂. The wet nanoparticles (NPs) was dried overnight in vacuum oven at 60°C. The
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52 nFe/Pd was analyzed by transmission electron microscope (TEM), scanning electron microscope
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54 integrated to energy-dispersive x-ray spectroscopy (SEM-EDS), and X-ray diffraction analysis
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56 (XRD). TEM analysis revealed the core-shell structure of NPs and size of Pd/nFe was around 60
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4 – 80 nm. XRD spectrum showed the single peak of Fe⁰ (110) at 2θ = 44.98°. The Pd coated NPs
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6 was observed by SEM-EDS with an average of 1 % Pd (wt/wt). The characterization results are
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8 shown in **Fig. S1**.
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10 11 12 13 14 15 2.3. Dechlorination of Aroclor 1248 by Pd-nFe and biodegradation by *Burkholderia xenovorans* 16 17 *LB400* 18

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21 Dechlorination of Aroclor 1248 was performed in 5ml vials under facultative anaerobic
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23 condition. Three micro liters (3 μl) of Aroclor 1248 stock solution in Toluene (50 mg mL⁻¹) was
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25 added to the vial and then air-dried to remove the solvent. Five milliliters of degassed distilled
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27 water and NPs (1 g L⁻¹) was added to each vial containing 150 μg Aroclor 1248. The vials were
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29 then sealed tightly by the Teflon cap. To disperse the NPs into solution, the vials were sonicated
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31 for 10s and rolled in a mixer at speed of 50 rpm for 6 days. The NPs were allowed to settle down
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33 at the bottom with a strong magnet. The supernatant was carefully transferred to a 50 mL flask.
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35 The efficiency of PCB dechlorination was determined by measuring the chloride ion released
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37 and the by-products by gas chromatography-mass spectrum analysis (GC-MS).
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44 The NPs treated sample was further subjected to biodegradation of the organic
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46 constituents under aerobic **condition** by *B. xenovorans*. For this purpose, MSM medium was
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48 added to the NP treated sample to bring it to a total volume of 10 ml. The samples were
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50 inoculated with growing cells of *B. xenovorans* LB400 with an initial OD_{600nm} of ~ 0.1 and
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52 incubated for 48h and the growth of *B. xenovorans* was monitored by measuring the turbidity at
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54 OD_{600nm}. The organic degradation products were analyzed by high-performance liquid
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56 chromatography (HPLC).
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8 *2.4 Analytical methods*
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10 The chloride ion release was quantified using an ion chromatograph (IC, Dionex DX-
11 120) equipped with Dionex IonPac AS-14 column and a conductivity detector. The eluting
12 solution was 3.5 mM Na₂CO₃ + 1 mM NaHCO₃ at a flow rate of 1.2 mL min⁻¹.
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18 Aroclor 1248 and dechlorinated products were analyzed by GC-MS. The compounds
19 were extracted by 1:1 v/v hexane:dichloromethane (Hex:DCM). The extraction was repeated
20 three times and the extracts were combined and dried gently under nitrogen gas. The extract was
21 re-dissolved in hexane to a volume of 1ml and transferred to GC vials. The samples were kept at
22 4°C before analysis. The samples and their dechlorinated intermediates were analyzed by Agilent
23 GC-MS 7890A/5975C system, equipped with a CTC autosampler (Agilent, Waldbronn,
24 Germany). The injector was set at 280°C and 1 µL of sample was injected using splitless mode.
25 A DB-5MS capillary column (60 m x 250 µm ID, 0.25 µm thickness; J&W Scientific, Folsom,
26 CA, USA) was used in combination with the GC oven temperature program as follows: 60°C (1
27 min), ramped 25°C min⁻¹ to 115°C and then increased by 1.5°C min⁻¹ to the final temperature of
28 240°C (20 min); total run time 106.3 min. The transfer line temperature was held at 280°C. The
29 ionization energy was 70 eV, the ion source and quadrupole temperatures were 250°C and
30 150°C, respectively. Samples before and after treatment were quantified in both selected ion
31 monitoring (SIM) for 32 single PCBs and full scan mode. PCB 209 was used as the internal
32 standard. 12 dioxin-like PCBs with coplanar structures selected from 32 congeners of sample set
33 were investigated to calculate the TEQ of mixture using SIM mode.
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58 The total PCB removal conversion ratio was determined as follows:
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$$\text{Removal PCB (\%)} = 100 - \frac{C_0 - C_{\text{remain}}}{C_0} \times 100$$

Total TEQ = $\sum_{i=1}^{12} (C_i * TEF_i)$; while C_i : individual PCB congener concentration

TEF: toxic equivalency factor developed by the World Health Organization (WHO) [27]

The samples from the biodegradation process were extracted with acetonitrile (1:3, v/v) and analyzed by HPLC using a C₁₈ column (Water Bondapak, 10 μm, 3.9 x 300 mm), mobile phase including 70% acetonitrile and 30% phosphoric acid (0.1%) with a flow rate of 1 ml min⁻¹. To define the metabolites, the mass spectrometry was performed in API 2000 system (Applied Biosystem, Foster City, CA) under the negative ion turbo spray ionization mode (ESI-LC/MS). The operating condition of system includes 4500V applied for the spray needle, declustering potential at 20V.

2.5. Toxicity evaluation

The toxicity of the samples were checked by using *Escherichia coli* DH5α. *E. coli* was added to samples consisting of only NP-control, NP treated PCB, bacteria-treated PCB and without any treatments (DI-control). The NP-control was prepared as follow: 500 mg L⁻¹ of NPs was anaerobically incubated in DI water for 24 h and then the NPs was removed by a strong magnet. The suspension was used to investigate the cytotoxicity in *E.coli*. After 24 h of exposure, the samples were tested for reactive oxygen species (ROS) level, the number of dead bacteria and glutathione peroxidase activity. Bacterial ROS level was determined by dichloro-dihydro-fluorescein diacetate (DCFH-DA) assay. The cell viability represented by amount of dead cells was checked by propidium iodine (PI) staining. The fluorescent intensity was

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4 measured by fluorescent spectrometer (Fluoromax 4 Spectrofluorometer, Horiba) and confocal
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6 laser scanning microscopy (Olympus FV3000 IX81) with excitation wavelength at 488nm
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8 (DCFH-DA) and 520 nm (PI), respectively. The glutathione peroxidase activity was determined
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10 colorimetrically using glutathione peroxidase activity assay kit (BioVision, Cat. #K762-100).
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18 **3. Results**

19 *3.1. Dechlorination of Aroclor 1248 by bimetallic nanoparticles Pd/nFe*

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21 The efficiency of the dechlorination process as a function of Cl⁻ release is shown in **Fig.**
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26 **1.** Chloride release occurred rapidly initially, but decreased to a slow rate thereafter. The rate of
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28 Cl⁻ release was ~2 ppm day⁻¹ and 89% of the total chloride was removed from the PCBs in about
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30 6 days, with a concurrent increase in the concentration of biphenyl. Aroclor 1248 comprised a
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32 mixture of mainly penta-, tetra-, and tri-chlorinated biphenyls (CBs) (**Fig. 2A**). After treatment
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34 with NPs, most of the chlorinated PCB congeners were dechlorinated, yielding biphenyl as the
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36 main byproduct (**Fig. 2B**). According to the results of GC-MS analysis of 32 congeners (**Table**
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38 **S1**), approximately 91% of the PCBs were dechlorinated after treatment with NPs. However, the
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40 32 PCB congeners comprised only 63.5% of the total PCB mass and the efficiencies measured in
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42 terms of total chloride released were not significantly different from those estimated by GC-MS
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44 analysis ; thus, the dechlorination capacity is presented in terms of total chloride released in this
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46 study. The removal of each PCB congener is shown in **Fig. 3**. The difficulty of dechlorination
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48 increased according to the number and position of the chlorines in the compound. For example,
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50 99% of tri-CBs were dechlorinated, whereas the dechlorination efficiencies of tetra-, penta-, and
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52 hexa- plus hepta-CBs were 92%, 84%, and 28%, respectively. In Aroclor 1248, the tri-, tetra-,
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4 and penta-CBs were the three main groups and comprised 97% of the total PCBs, which suggests
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6 that pretreatment with NPs resulted in high efficiency dechlorination of Aroclor 1248.
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10 11 12 13 3.2. Aerobic biodegradation of Aroclor 1248 after treatment with NPs 14

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16 The results of aerobic biodegradation of NP-treated Aroclor by *B. xenovorans* LB400 are
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18 shown in **Figs 4A** and **4B**. The bacterium metabolized biphenyl, and HPLC analysis showed that
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20 the degradation products included benzoic acid and *cis,cis*-muconic acid, with retention times of
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22 1.9 and 6.2 min, respectively. ESI-LC/MS analysis confirmed the presence of these compounds
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24 based on the presence of [M-H]⁻ peaks with *m/z* values of 120.7 and 140.7, respectively (**Fig.**
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26 **S2**). The concentration of benzoic acid increased and reached 6.0 ppm after 36 h, before
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28 declining to 3.9 ppm within 48 h (**Fig. 4B**). Bacterial growth was confirmed by measuring an
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30 increase in the OD_{600nm} from 0.09 to 0.125. The concentration of biphenyl decreased
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32 significantly to about 90% after 24 h.
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42 3.3. Evaluation of the comparative toxicity levels of PCBs using *Escherichia coli* 43 44

45 TEQ values were used as a toxicity indicator for Aroclor 1248 before and after treatment.
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47 The toxicity of Aroclor 1248 is shown for 12 of its dioxin-like congeners (**Table 1**), which were
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49 selected from an analysis of 32 congeners, as shown in **Fig. 3** and **Table S1**. Low TEQ
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51 congeners such as PCB 77 and 81 were removed very efficiently, i.e., 93.5% and 98.8%,
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53 respectively, whereas high TEQ congeners such as PCB 105, 126, and 156 were removed 6.7%,
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55 33.6%, and 77.7%, respectively. Overall, **Table 1** shows that the TEQ values of the samples
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57 treated with NPs decreased from 33.8×10^{-5} to $9.5 \times 10^{-5} \mu\text{g g}^{-1}$.
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4 Cytotoxic effects in *E. coli* are indicated by reduced cell viability, an increased level of
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6 reactive oxygen species (ROS), and an increase in glutathione peroxidase activity. In this study,
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8 dead bacteria were detected by measuring the intensity of PI staining, and ROS levels were
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10 determined by DCFH-DA assay. We found that there was a strong correlation between the
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12 increase in the number of dead bacteria and the ROS level, as shown in **Fig. 5A** and **5B**,
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14 indicating that the samples containing Aroclor 1248 were toxic before treatment and that the NP-
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16 control had the highest toxicity level. The NP-control sample resulted in a higher stress level
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18 than Aroclor 1248 before it was treated with NPs. Before treatment, the ROS level induced by
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20 Aroclor 1248 was 5.25-fold higher than that in the DI-control without any treatment, while the
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22 ROS level was only 3.78- and 2.39-fold higher in the samples containing PCBs after
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24 dechlorination (NP-treated) and subsequent biodegradation (bacteria-treated), respectively. The
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26 glutathione peroxidase activity also increased significantly; glutathione peroxidase activities
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28 were 0.3 mU for the DI-control, 9.4 for Aroclor 1248 before treatment, and 3.2 and 2.6 mU after
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30 treatment with NPs and bacteria, respectively (**Fig. 5C**).
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42 **4. Discussion**

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45 In this study, we found that bimetallic nanoparticles Pd/nFe was effective for the
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47 dechlorination of a mixture of PCBs, Aroclor 1248, and that the dechlorinated organic phenolic
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49 compounds were readily biodegraded to innocuous products. The strengths of the effects of
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51 chlorine substitution (number and location) in the carbon skeleton in PCB on the efficiency and
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53 selectivity of the dechlorination activity of NPs were ranked previously in the following order:
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55 ortho- (o-) > meta- (m-) > para- (p-) [28, 29]. This was also the case for Aroclor 1248 in the
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57 present study (**Table S1**).
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4 In our study, we observed a lower efficiency of reductive dechlorination of Aroclor 1248
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6 using bimetallic NPs than Zhang et al. [20], who reported that 95.8% of Aroclor 1242 was
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8 dechlorinated by Ni/nFe after 24 h. This is in contrast to the 89% of total chlorine removed from
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10 Aroclor 1248 by Pd/nFe after a 6-day treatment in the present study. Several factors could
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12 determine the efficiency of Aroclor dechlorination. Aroclor 1242 and 1248 both contain similar
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14 amounts of chlorine, but di-, tri- and tetra-CBs are the main groups in Aroclor 1242, whereas tri-
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16 , tera-, and penta-CBs are the main groups in Aroclor 1248. The metal ion doped on the surface
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18 of iron as well as size of particles could also affect the efficiency of dechlorination. For example,
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20 Lowry et al. [18] showed that PCB dechlorination has not been significantly promoted by using
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22 microscale ZVI at the ambient condition. He also compared the dechlorination rates between
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24 samples treated with palladized micro zero-valent iron (ZVI) and with unpalladized nano-sized
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26 ZVI. Although Pd/micro ZVI could promote a faster rate of dechlorination, the enhancement was
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28 a short lived and cost effect should be considered. In addition, Schrick et al. [30] showed that
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30 Pd/nFe could reduce trichloroethylene (TCE) faster than Ni/nFe, however it had a higher
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32 corrosion rate. Furthermore, reductive dechlorination using bimetallic NPs as a catalyst resulted
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34 in a lower remediation time than when a mixture of bacteria was used [11, 12, 31, 32]. Zhen et
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36 al. [12] found that a mixed culture containing *Dehalococcoides mccartyi* strain 195 dechlorinated
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38 42% of Aroclor 1260 within 250 days in the presence of 1,2,3,4-tetrachlorobenzene. Similar
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40 results were reported by Wang and He [11] who reported the removal of > 50% of the hepta- and
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42 hexa-CB congeners from Aroclor 1260 after 4 months using mixed cultures of *Dehalococcoides*
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44 and *Dehalobacter*.

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56 PCB biodegradation by ortho-directed strains such as *Burkholderia* sp. LB400 or
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58 *Rhodococcus* sp. RHA1 preferentially oxidize ortho-chlorinated PCB rings [33, 34] to produce
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4 readily degradable PCB congeners. *Burkholderia* sp. LB400 has a low capacity to degrade PCB
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6 congeners that contain chlorine at the *p*- position, such as PCB15 and PCB66, whereas it has a
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8 higher capacity to degrade congeners containing chlorine at both the *p*- and *o*- positions, such as
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10 in PCB 47 [17]. In this study, *B. xenovorans* was used after the dechlorination step to further
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12 mineralize the Aroclor. However, benzoic acid and chlorobenzoate were still detected, but these
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14 chlorinated compounds are less persistent in the environment than their PCB parent compounds.
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16 Because biphenyl was the dominant intermediate, benzoic acid and its metabolite, *cis,cis*-
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18 muconic acid, were detected as intermediate products. A rapid and almost complete
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20 biodegradation of biphenyl by *B. xenovorans* was observed. To confirm the degradation of
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22 benzoic acid by this bacterium, it was grown in MSM containing 10 ppm benzoic acid as the sole
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24 carbon source. The results showed that it was metabolized completely within 48 h.
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32 Recently, sequential anaerobic and aerobic bacterial treatment of PCBs in sediments have
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34 been reported by Payne et al.[32]. Although it is a sustainable strategy, the requirement of long
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36 time remediation (120 days) caused by slow activity of dehalorepiring bacteria as well as toxicity
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38 of PCB congeners, such as PCB 126 or PCB 169 could be a limitation factor. He et al.[35]
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40 reported the potential use of a sequential model consisting of Pd/Fe and aerobic bacterium in
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42 PCB 101 remediation. They showed that the nano/bio treatment could be a feasible method in
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44 remediation of PCB contaminated soil. However, He et al., [35] observed lower removal
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46 efficiency of single PCB in comparison to our study which showed a nearly complete removal of
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48 the commercial PCB mixture.
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54 The toxicity of PCB mixtures has been a major concern for the last several decades.
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56 Assessments of their toxic equivalency factors (TEFs) in male Wistar rats showed that the toxic
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58 potentials of PCBs, in terms of ethoxyresorufin O-deethylase induction, increased in the
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4 following order: Aroclor 1254 > Aroclor 1248 > Aroclor 1242 > Aroclor 1260 > Aroclor 1232
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6 [36]. There were good correlations between the observed and calculated ED₅₀ values using the
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8 conservative TEFs. Zhang et al. [37] measured the relative TEQ values of various Aroclors using
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10 a luciferase reporter gene assay in chickens, ring-necked pheasants, and Japanese quail, and
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12 found that Aroclor 1248 had a higher toxicity than Aroclors 1242, 1254, 1260, 1016, and 1221.
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14 Although there is considerable controversy over the accuracy of TEQ in determining the PCB
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16 toxicity, TEQ is still used widely to estimate the toxicity of non- and mono-ortho PCBs in biota.
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18 Prignamo et al. [9] determined the toxicity of several PCB mixtures and found that the TEQ of
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20 Aroclor 1248 at 11.7% was higher than those of Aroclor 1254 and 1260 at 7.7%, and 1%,
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22 respectively, which contain highly chlorinated congeners of PCB and a high percentage of
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24 chlorine.
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32 We used TEQ to validate the toxicity level of Aroclor 1248 before and after treatment.
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34 The TEQ values decreased after treatment and correlated well with the results of the stress
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36 exposure assay using *E. coli*. And, ROS levels and glutathione peroxidase enzyme activity are
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38 often used to evaluate stress in biological samples, and in this study, they demonstrated that the
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40 toxicity of Aroclor 1248 decreased after treatment. However, low toxicity effects on *E.coli* were
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42 still observed in bacteria-treated Aroclor1248 samples, which may be due to the presence of low
43
44 concentrations of dioxin-like PCB congeners, the co-effect of NPs, or the impact of growth
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46 medium components. The effects of Aroclor 1248 and other treatments on *E. coli* cell viability
47
48 and cell morphology were also investigated. *E. coli* viability (**Fig. 5A, S3A**) and ROS assays
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50 (**Fig. 5B, S3C**) based on fluorescent staining measurements, including spectrometry and confocal
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52 microscopy, showed that untreated Aroclor 1248 had high toxicity toward *E. coli*. The activity of
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54 glutathione peroxidase which can reduce lipid peroxidation, was higher in samples that contained
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4 organic pollutants than in NP-control samples, which may be explained by higher toxicity of
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6 PCBs. Katynski et al. [38] determined that PCBs and other aryl hydrocarbon receptor agonists
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8 could induce membrane lipid peroxidation and change membrane fluidity in chicken embryos.
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10 Hence, the stronger lipid peroxidation could be a reason for temporarily increased activity of
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12 glutathione peroxidase in untreated Aroclor 1248 sample. Besides, we found that Aroclor 1248
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14 led to bacterial membrane damage and the release of cellular contents resulting from the
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16 aggregation of the damaged cells (highlighted by the circled regions) which was detected as
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18 morphological changes in *E. coli* (Fig. S3B). Under light microscopy, *E. coli* exposed to Aroclor
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20 1248 before treatment died and aggregated to form large clusters, whereas *E. coli* exposed to
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22 Aroclor 1248 after treatment formed only very small aggregates or separated homogeneously in
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24 suspensions that contained dead or live cells.
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32 The NP-control sample also induced intracellular stress in *E. coli*, but the low activity of
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34 glutathione peroxidase associated with the NPs indicated that the bacterial lipid membrane was
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36 weakly affected by the NPs. Gunawa et al. [39] found that although the ROS level increased in
37
38 the presence of NPs, oxidative stress dropped rapidly as a result of the induction of biological
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40 defense systems. The ions released from NPs, including niken or aluminum, could be toxic to
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42 bacteria affecting biodegradation activities [40]. Moreover, Kim et al. [41] reported that
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44 palladized nFe had the least toxic effect compared with other Fe-based NPs, including the bare
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46 form. Given that less toxic intermediates are formed by PCB degradation, the results of the
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48 present study demonstrate that our nano-bio hybrid treatment method could be a promising
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50 system for the remediation of persistent organic pollutants.
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60 **5. Conclusions**

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4 The catalytic dechlorination of Aroclor 1248 using bimetallic NPs (Pd/nFe) was
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6 performed under mild conditions, namely at room temperature and at pH ~ 6, and under
7
8 anaerobic conditions. A high efficiency of dechlorination was observed and less toxic
9
10 intermediates were produced. After the treatment of Aroclor 1248 with NPs, biodegradation
11
12 yielded less toxic and mostly innocuous compounds. The use of a combined chemical and
13
14 biological treatment strategy has many advantages over a sequential anaerobic/aerobic
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16 biotreatment process, and in addition appreciably reduces the treatment period which is
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18 considerably long in the case of sequential treatment methods.
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27 **Acknowledgments**

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33 of Environment (RE201402059).
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4 **Figure Legends:**
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9 **Figure 1.** Chloride release and biphenyl production during dechlorination of Aroclor 12480 with
10 bimetallic Pd/nFe.
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12 **Figure 2.** Gas chromatogram of Aroclor 1248 before (A) and after (B) NP treatment. Biphenyl
13 was the main intermediate identified.
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16 **Figure 3.** Concentration of the dominant PCB groups, tri-, tetra-, pen-, and hexa-CB, remaining
17 after Pd/nFe treatment
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20 **Figure 4.** HPLC analysis of degradation products (i.e., benzoic acid and *cis,cis*-muconic acid)
21 after biodegradation of NP-treated Aroclor 1248 (A). Growth of bacteria (OD), and degradation
22 of biphenyl and benzoic acid (B).
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28 **Figure 5.** Evaluation of Aroclor 1248 cytotoxicity in *E.coli* before and after NP-(Pd/nFe)
29 treatment. *E.coli* was treated with samples containing only DI water (DI -control), Aroclor 1248
30 before treatment (before treatment), NP-control (Pd/nFe), NP-treated PCBs (NP-treated), or
31 PCBs after treatment with the nano-bio hybrid system (bacteria-treated).
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Figure 1

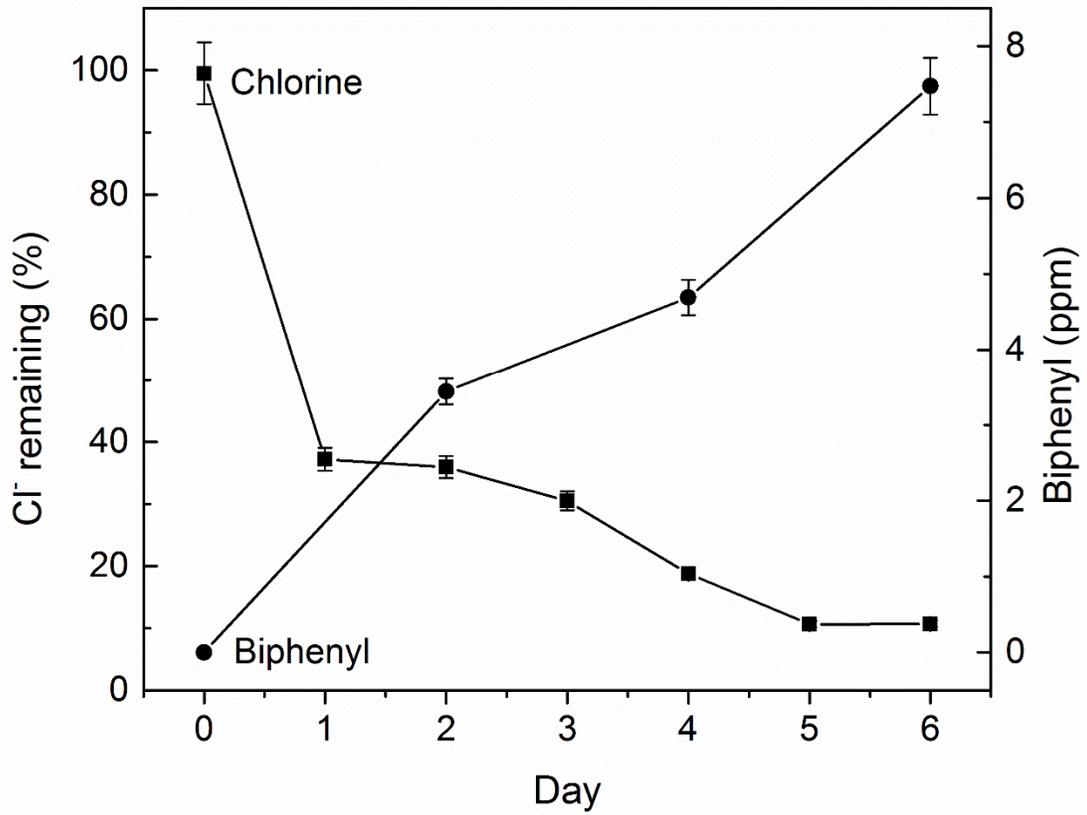


Figure 2

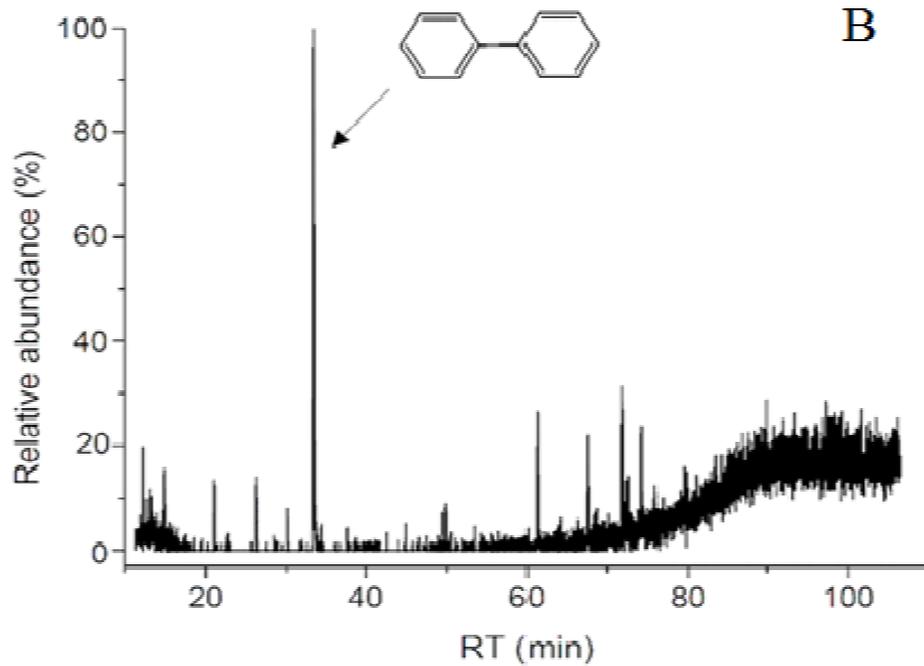
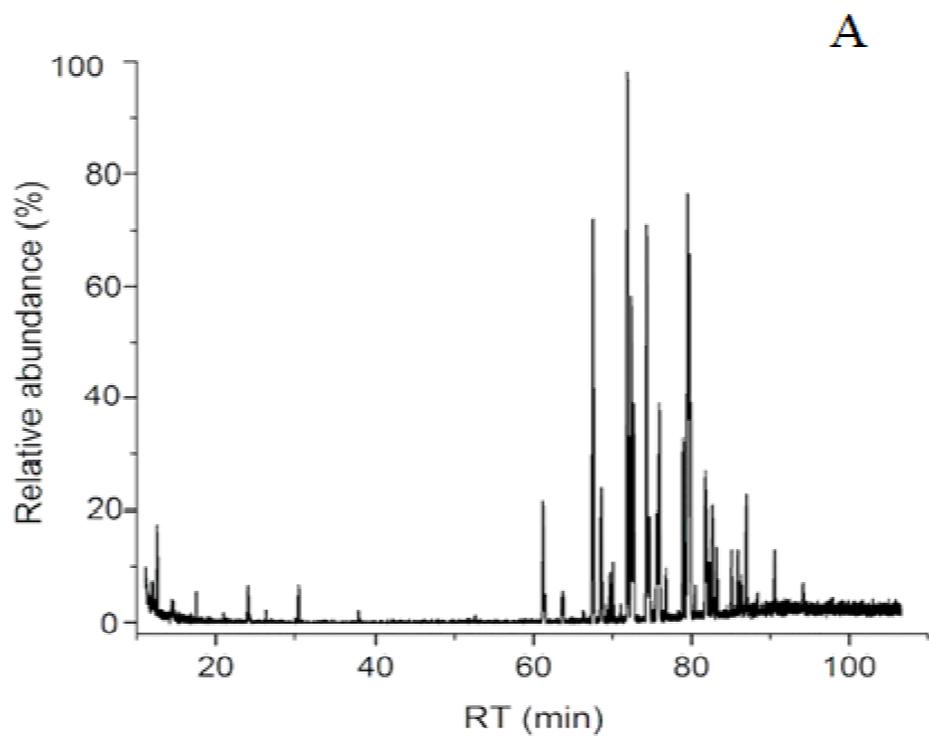


Figure 3

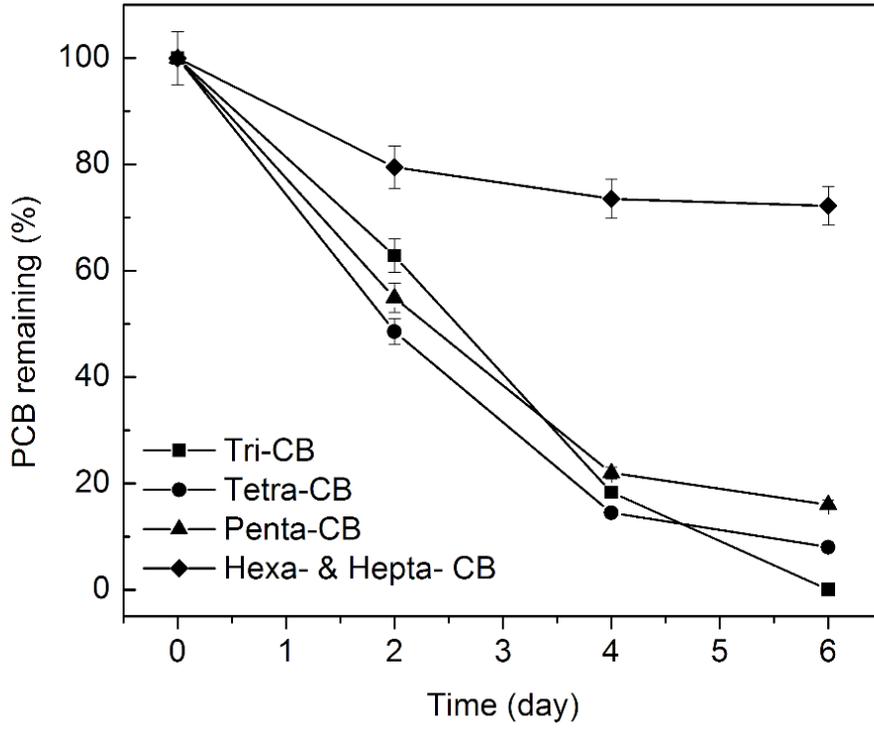
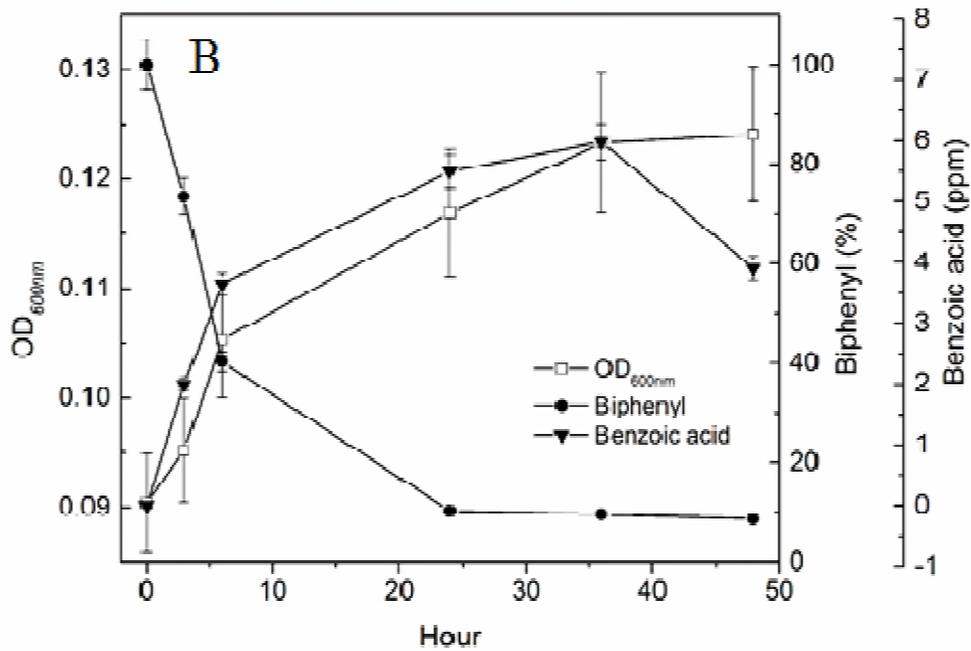
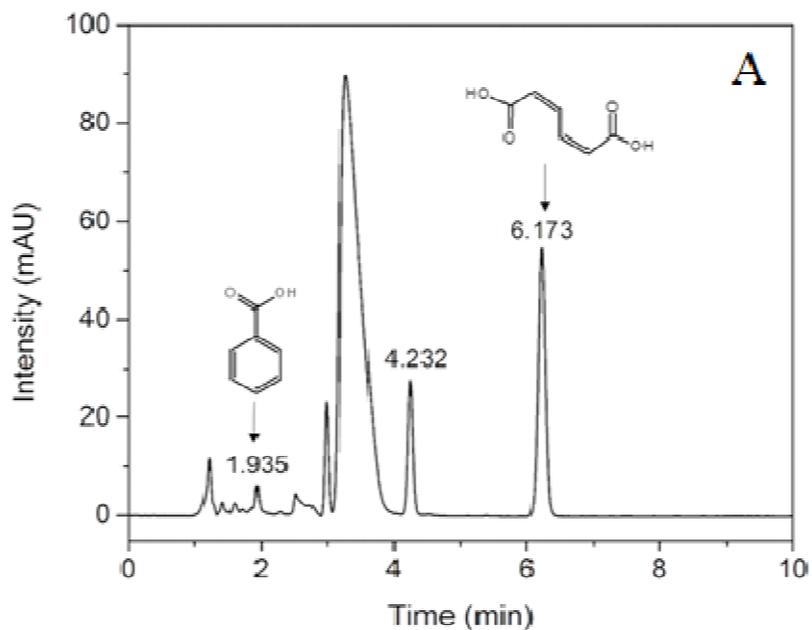


Figure 4



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7 **Figure 5**

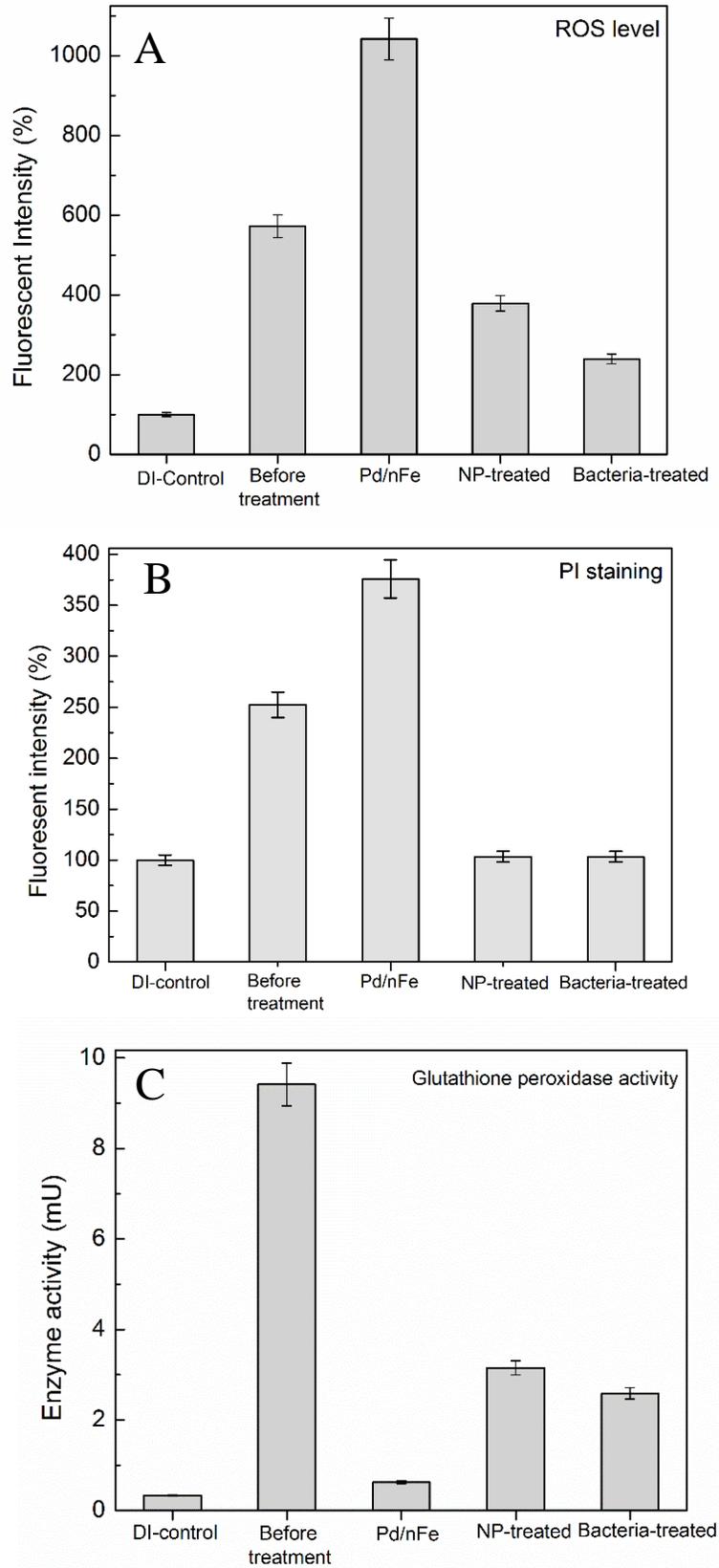


Table 1: TEQ values of Aroclor 1248 before and after dechlorination with bimetallic NPs Pd/nFe

PCB congener	PCB remaining (%)	TEQ ($\mu\text{g g}^{-1}$)	
		Before	After
77	6.5	1.22e^{-5}	7.9e^{-7}
81	1.2	1.8e^{-5}	2.1e^{-7}
105	26.7	1.08e^{-6}	2.9e^{-7}
114	4.5	1.44e^{-5}	6.5e^{-7}
118	5.9	2.06e^{-5}	1.2e^{-6}
123	9.7	6.3e^{-7}	6.1e^{-8}
126	33.6	2.7e^{-4}	9.1e^{-5}
156	77.7	3.6e^{-7}	2.8e^{-7}
157	86.3	1.41e^{-7}	1.2e^{-7}
167	60.4	9e^{-8}	5.4e^{-8}
169	87.7	1.74e^{-7}	1.5e^{-7}
189	84.6	9e^{-9}	7.6e^{-9}
Total TEQ		33.8e^{-5}	9.5e^{-5}

Supplementary materials

Nano/bio treatment of polychlorinated biphenyls with evaluation of comparative toxicity

comparative toxicity

Thao Thanh Le^{a#}, Hoang Khanh Nguyen^{a#}, Jong-Rok Jeon^b, Arokiasamy J. Francis^c, Yoon-Seok Chang^{a*}

^aSchool of Environmental Science and Engineering, POSTECH, Pohang 790-784, Republic of Korea

^bCorporate R&D, LG Chem Research Park, Daejeon 305-380, Republic of Korea

^cDivision of Advanced Nuclear Engineering, POSTECH, Pohang 790-784, Republic of Korea; and Environmental Sciences Department, Brookhaven National Laboratory, Upton, New York, USA

Authors have the same distribution

*** Corresponding author**

Prof. Dr. Yoon-Seok Chang

School of Environmental Science and Engineering,
Pohang University of Science and Technology (POSTECH),
Pohang 790-784, Republic of Korea

Tel.: +82 54 279 2281; Fax: +82 54 279 8299

E-mail address: yschang@postech.ac.kr (Y.-S. Chang)

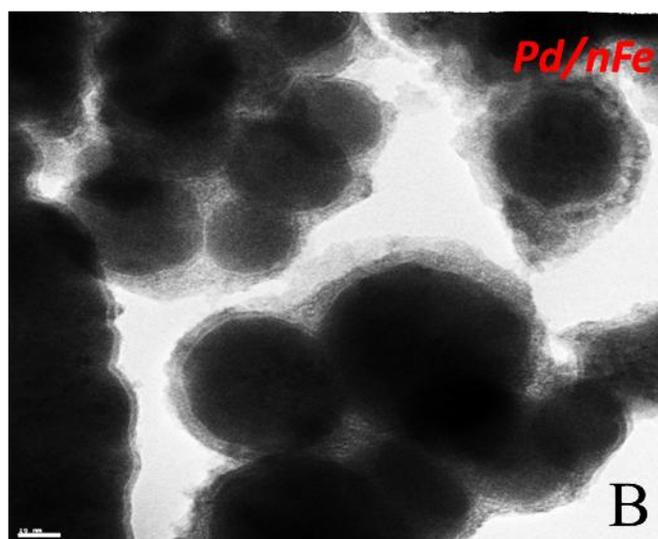
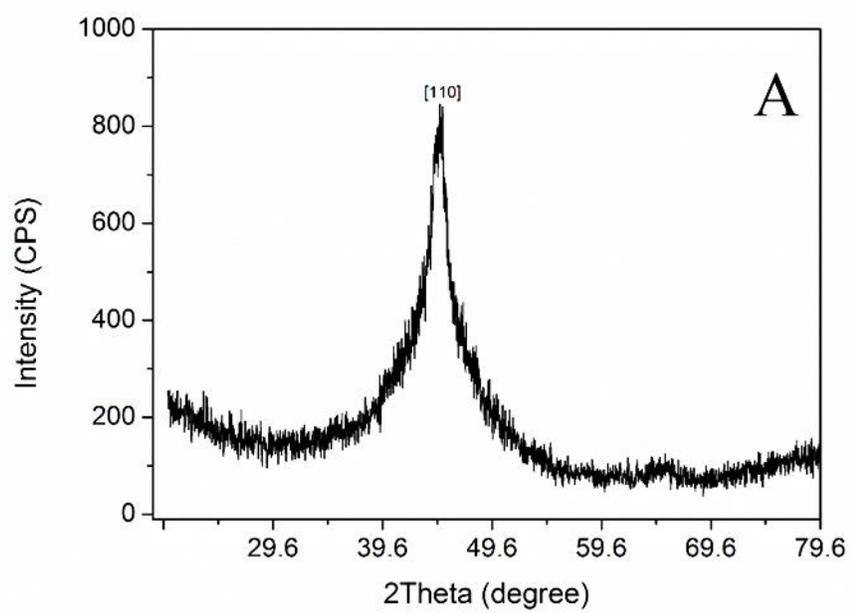


Fig. S1: Characteristics of bimetallic NPs Pd/nFe, including their XRD (A) and TEM (B)

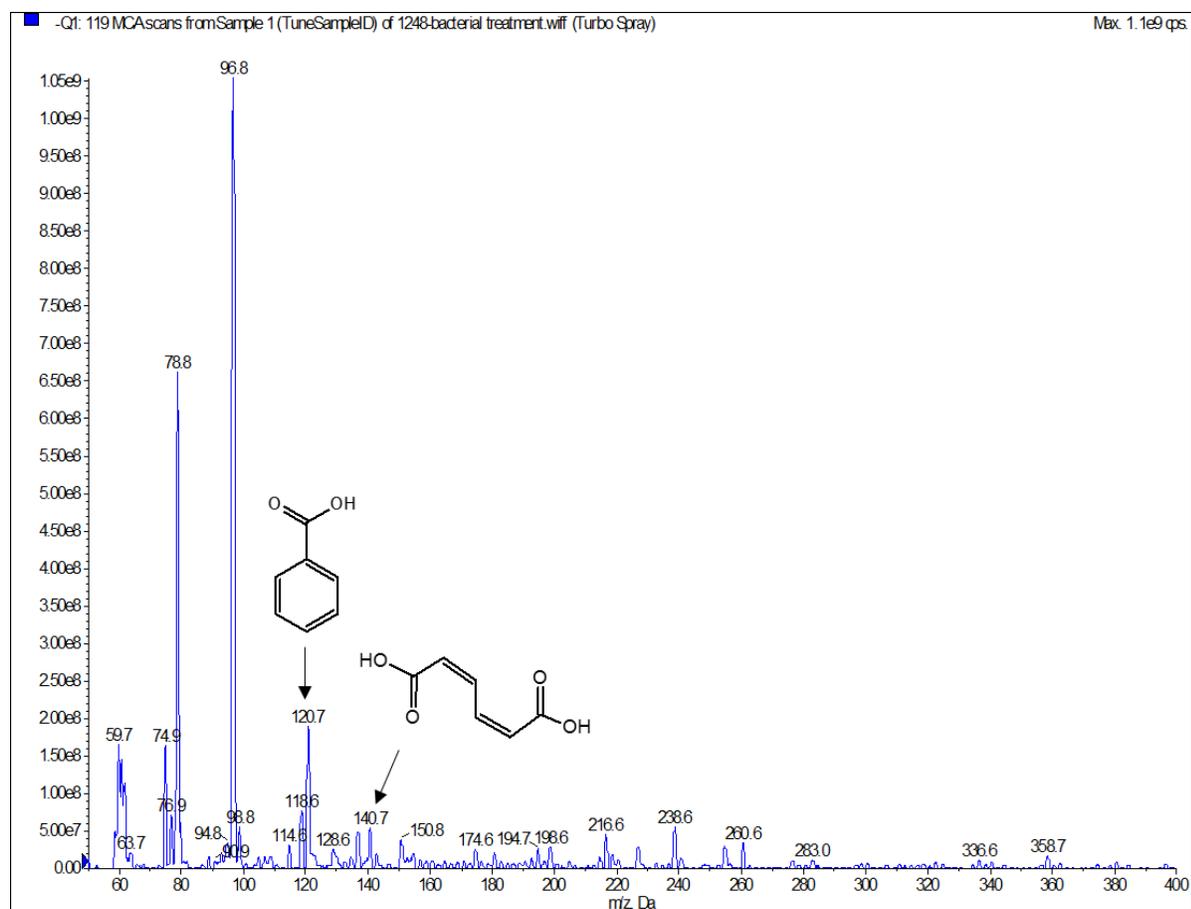


Fig. S2: MS chromatograph of a PCB solution after biodegradation. Benzoic acid and *cis,cis*-muconic acid were identified by $[M-H]^-$ peaks at m/z 120.7 and 140.7, respectively.

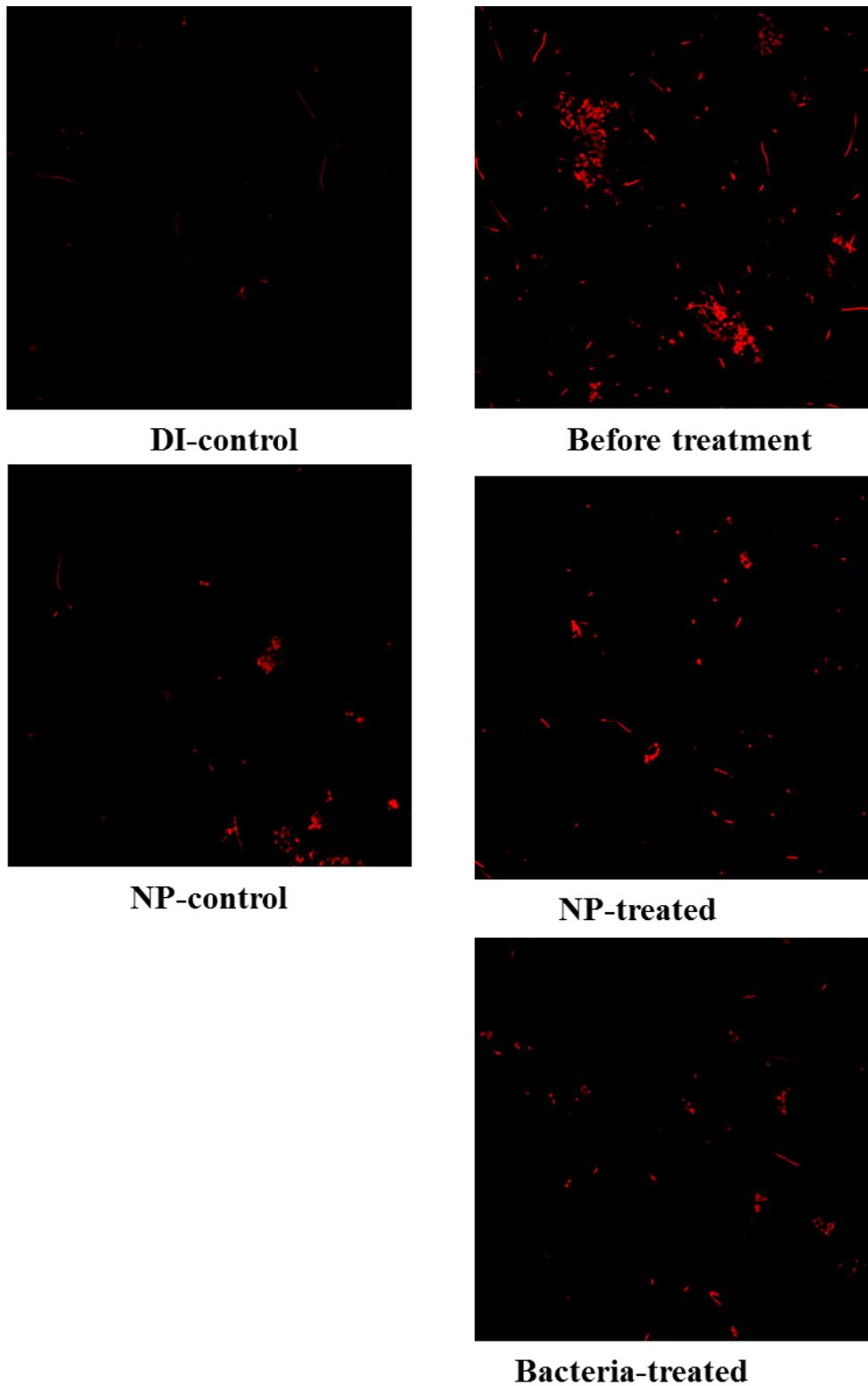


Fig. S3A: Cytotoxicity during the stress exposure: Dead bacteria detected by PI signals (Red) under confocal microscopy

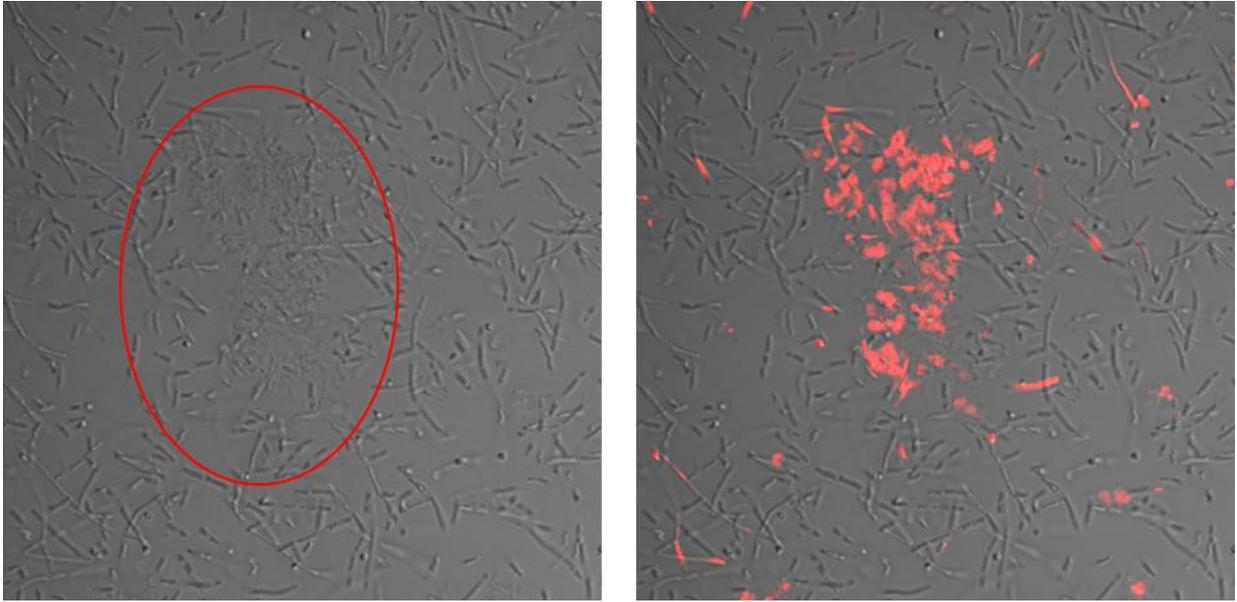


Fig. S3B: Cytotoxicity during the stress exposure: changes in cell morphology

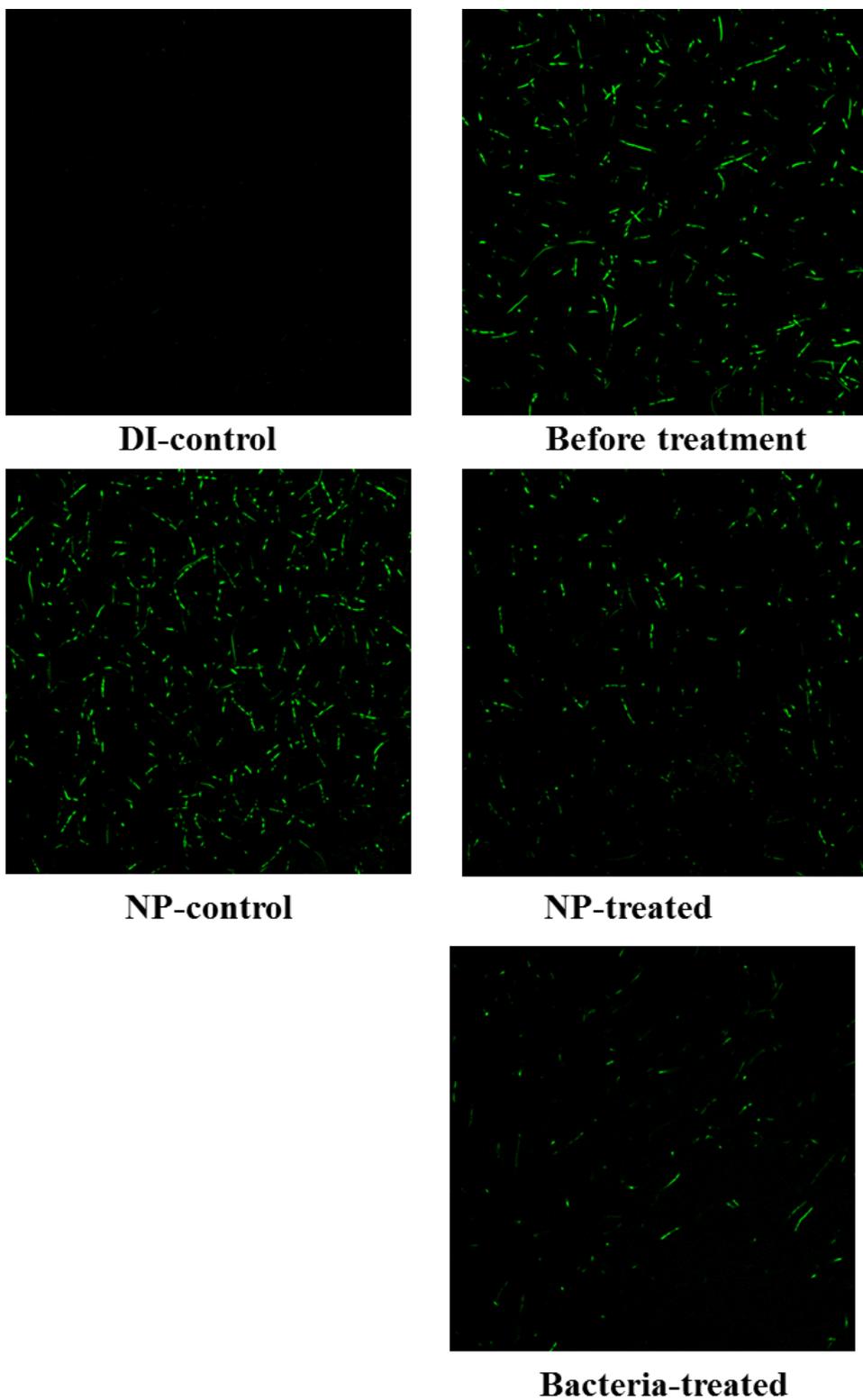


Fig. S3C: Cytotoxicity during the stress exposure: ROS level observed by DCFH-DA signals (Green) under confocal microscopy

Table S1: PCB removal efficiencies and chemical structure of 32 PCB congeners

PCB congener	Removal (%)	No. Chlorine			Angular (°)	
		o-	m-	p-		
Tri-CB	19	94.4	3	0	0	89.3
	31	97.3	1	1	1	51.7
	33	98.6	1	1	1	56
	37	99.6	0	1	2	36.4
Tetra-PCB	40	89.5	2	2	0	90.5
	44	90.0	2	2	0	79.9
	49	89.5	2	1	1	80.5
	52	87.5	2	2	0	103
	54	85.1	4	0	0	90
	56	90.6	1	2	1	59.1
	60	89.7	1	1	2	90.9
	64	88.3	2	1	1	89.7
	66	91.6	1	1	2	54.4
	70	91.2	1	2	1	51.9
	74	91.4	1	1	2	52.9
	77	93.5	0	2	2	142
	81	98.8	0	2	2	141.5

	PCB congener	Removal (%)	No. chlorine			Angular (°)
			o-	m-	p-	
	87	71.5	2	2	1	91.2
	95	67.4	3	2	0	90.5
	99	74.2	2	1	2	98.9
	105	73.3	1	2	2	121.1
Penta-CB	110	75.3	2	2	1	78.1
	114	95.5	1	2	2	90.9
	118	94.1	1	2	2	50.9
	123	90.3	1	2	2	51.6
	126	66.4	0	3	2	36.8
	156	22.3	1	3	2	55.2
	157	13.7	1	3	2	91
Hexa-CB	167	39.6	2	2	2	50.7
	169	12.3	0	4	2	142
	188	15.4	4	2	1	90
Hepta-CB	189	23.1	1	4	2	90.8

Abstract

The persistence of polychlorinated biphenyl (PCB) Aroclor 1248 in soils and sediments is a major concern because of its toxicity and presence at high concentrations. In this study, we developed an integrated remediation system for PCBs using chemical catalysis and biodegradation. The dechlorination of Aroclor 1248 was achieved by treatment with bimetallic nanoparticles Pd/nFe under anoxic conditions. Among the 32 PCB congeners of Aroclor 1248 examined, our process dechlorinated 99%, 92%, 84%, and 28% of tri-, tetra-, penta-, and hexachlorinated biphenyls, respectively. The resulting biphenyl was biodegraded rapidly by *Burkholderia xenovorans* LB400. Benzoic acid was detected as an intermediate during the biodegradation process. The toxicity of the residual PCBs after nano-bio treatment was evaluated in terms of toxic equivalent values which decreased from $33.8 \times 10^{-5} \mu\text{g g}^{-1}$ to $9.5 \times 10^{-5} \mu\text{g g}^{-1}$. The residual PCBs also had low cytotoxicity toward *Escherichia coli* as demonstrated by lower reactive oxygen species levels, lower glutathione peroxidase activity, and a reduced number of dead bacteria.