

BIODEGRADATION OF THE ULTRAVIOLET FILTER BENZOPHENONE-3  
UNDER DIFFERENT REDOX CONDITIONSYOU-SHENG LIU,<sup>†‡</sup> GUANG-GUO YING,<sup>\*†‡</sup> ALI SHAREEF,<sup>‡</sup> and RAI S. KOOKANA<sup>‡</sup><sup>†</sup>State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou, P.R. China<sup>‡</sup>CSIRO Land and Water, Water for a Healthy Country Flagship, Glen Osmond SA, Australia

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**Abstract**—Biodegradation of the ultraviolet (UV) filter benzophenone-3 (BP-3) was investigated in the laboratory to understand its behavior and fate under oxic and anoxic (nitrate, sulfate, and Fe [III]-reducing) conditions. Biodegradation experiments were conducted in microcosms with 10% of activated sludge and digested sludge under oxic and anoxic conditions, respectively. Benzophenone-3 was well degraded by microorganisms under each redox condition. Under the redox conditions studied, the biodegradation half-life for BP-3 had the following order: oxic (10.7 d) > nitrate-reducing (8.7 d) > Fe (III)-reducing (5.1 d) > sulfate-reducing (4.3 d) ≥ anoxic unamended (4.2 d). The results suggest that anaerobic biodegradation is a more favorable attenuation mechanism for BP-3. Biodegradation of BP-3 produced two products, 4-cresol and 2,4-dihydroxybenzophenone, under oxic and anoxic conditions. Biotransformation of BP-3 to 2,4-dihydroxybenzophenone by way of demethylation of the methoxy substituent (*O*-demethylation) occurred in cultures under each redox condition. The further biotransformation of 2,4-dihydroxybenzophenone to 4-cresol was inhibited under oxic, nitrate-reducing, and sulfate-reducing conditions. Environ. Toxicol. Chem. 2012;31:289–295. © 2011 SETAC

**Keywords**—UV filter Biodegradation Oxic Anoxic Sludge

## INTRODUCTION

Ultraviolet (UV) filters have been used in consumer sunscreen products in amounts up to 10% and also in many other personal care products including skin creams, cosmetics, hair sprays, body lotions, hair dyes, and shampoos [1]. The consumption of UV filters is increasing because of the growing awareness of hazards posed by UV radiation and recommendations for prevention of skin cancer. Benzophenone-3 (BP-3), also known as oxybenzone or 2-hydroxy-4-methoxy benzophenone, is a commonly used UV filter in cosmetic formulations such as sunscreens and skin care products worldwide [2,3]. Widespread use of BP-3 in personal care products was documented in a survey in the U.S. population [4]. Consequently, BP-3 reaches aquatic environments directly through recreational activities (swimming and bathing) and indirectly by way of wastewater treatment plants (WWTPs) because of incomplete removal [2,5,6]. It is reported that BP-3 showed hormonal activities (estrogenic, antiestrogenic, androgenic, and antiandrogenic) in fish in both in vitro and in vivo tests [7–9]. In male medaka and male California halibut BP-3 lead to vitellogenin induction [10,11].

Benzophenone-3 has been detected in untreated municipal wastewaters at concentrations between 720 ng/L and 7800 ng/L [2], in treated wastewaters at concentrations of 10 to 700 ng/L [2,12], in water of some Swiss lakes at concentrations of <2 to 125 ng/L [2,13]. Because of its hydrophobic properties, BP-3 has been found also in solid matrices and biota at concentrations of 10 to 20 ng/g in sewage sludge [14] and 3 to 21 ng/g in fish [15]. In the aquatic environment, BP-3 may affect the natural attenuation (e.g., photolysis) of other photosensitive organic

contaminants [16]. Therefore, a sound understanding of the attenuation mechanisms of BP-3 in the activated treatment process of WWTPs and natural aquatic environment during biodegradation is desirable.

Previous studies have shown that BP-3 could be eliminated significantly during wastewater treatment, which is mainly driven by both sorption onto sewage sludge and aerobic biodegradation [2,6]. Removal efficiencies in conventional WWTPs have been reported to be in the range of 68 to 96% for BP-3 [2,6]. Benzophenone-3 could absorb UV-B (290–320 nm) or UV-A (320–400 nm) because it is designed to protect from harmful UV light; thus, it is quite stable under UV exposure. Rodil et al. [16] demonstrated a high stability of BP-3 during a whole irradiation period of 72 h under artificial sunlight radiation. Therefore, biodegradation could be the main attenuation mechanism for BP-3 in the natural environment. Microbial respiration in aquatic systems has been widely reported to take place by way of a variety of electron acceptors [17,18]. Microbial degradation of organic compounds through microbial respiration processes can be influenced by the type of electron acceptors present in the aquatic environment [19–21]. So far, little information has been available in the literature on the fate of BP-3 in the environment, especially its biodegradation potential and mechanisms under various redox conditions.

The objective of the present study was to investigate the biodegradation efficiency of one representative UV filter, BP-3, in the laboratory under oxic and anoxic conditions (nitrate-reducing, sulfate-reducing, and Fe [III]-reducing conditions). Because BP-3 is the most commonly detected UV filter in wastewaters, its tentative biotransformation pathways under each redox condition were explored further. Fresh activated sludge and anaerobic digested sludge were used as inocula for aerobic and anaerobic microcosms, respectively, to understand the fate of BP-3 in the WWTPs and environment. Various reducing conditions were created to study the effect of electron acceptors on biodegradation of BP-3 by using the media

All Supplemental Data may be found in the online version of this article.

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amended with nitrate, sulfate, or Fe (III) under anoxic conditions.

## MATERIALS AND METHODS

### Chemicals

High-purity BP-3 (98%) and benzylcinnamate (99%, internal standard) were purchased from Dr. Ehrenstorfer. Benzo-phenone-3 has nonpolar and weakly basic properties with  $pK_a$  7.56,  $pK_{ow}$  3.79, and Henry's law constant  $1.50 \times 10^{-8}$  (atm m<sup>3</sup>/mol). Iron (III) citrate, resazurin, Na-L lactate, and NaN<sub>3</sub> of analytical grade were obtained from Sigma-Aldrich. Sodium nitrate, Na<sub>2</sub>SO<sub>4</sub>, and Na<sub>2</sub>S of analytical grade were obtained from BDH. High-performance liquid chromatography grade methanol and dichloromethane were purchased from Sigma-Aldrich. A stock solution (1,000 mg/L) of BP-3 was prepared in methanol. All glassware was hand-washed with tap water, rinsed with high-performance liquid chromatography grade water and methanol, and baked at 450°C for at least 4 h before use.

### Microcosms

Aerobic and anaerobic microcosms were prepared by using freshly obtained activated sludge and digested sludge as inocula, respectively, from a WWTP in Adelaide, South Australia. Incubation solutions with 10% of each inoculum (v/v) were prepared in minimal salts medium consisting of KCl (1.3 g/L), KH<sub>2</sub>PO<sub>4</sub> (0.2 g/L), NaCl (1.17 g/L), NH<sub>4</sub>Cl (0.5 g/L), CaCl<sub>2</sub> · 2H<sub>2</sub>O (0.10 g/L), MnCl<sub>2</sub> · 6H<sub>2</sub>O, and NaHCO<sub>3</sub> (2.8 g/L) [22] and amended with trace salts and vitamins [23]. For anoxic treatment, the medium was deoxygenated by boiling with nitrogen gas for 15 min and cooled in an anaerobic chamber.

### Biodegradation experiments

The biodegradation experiments were conducted as follows. Two 1-L glass Schott bottles with 500 ml media were set up for each treatment of oxic and anoxic-nitrate-reducing, sulfate-reducing, and Fe (III)-reducing conditions. Sterile controls for each treatment were included. The test compound was spiked into the incubation media at 1 mg/L in each treatment by pipetting 0.5 ml each of BP-3 stock solution. For the sterile controls of each treatment the bottles containing the spiked media were autoclaved (120°C, 20 min) for three consecutive days followed by the addition of the metabolic inhibitor sodium azide (NaN<sub>3</sub>, 0.5% final concentration) to maintain sterility. Two replicate samples from each treatment were collected at predetermined sampling time intervals (0, 1, 3, 7, 10, 14, 18, 21, 28, 35, and 42 d following treatment).

### Oxic treatment

In the batch test, 500 ml incubation solutions (10% of the aerobic inoculum, v/v) prepared in 1-L Schott bottles as described above were incubated at 25°C in an orbital mixer incubator (Ratek OM11) with continuous shaking at 300 rpm. Oxic condition was maintained by opening the caps three times a day in a laminar flow chamber.

### Anoxic treatments

All sample preparations for anoxic treatments were conducted in an atmosphere of N<sub>2</sub>/CO<sub>2</sub> (80:20, v/v), inside an anaerobic chamber. One-liter bottles containing 500 ml incubation solutions with 10% of the anaerobic inoculum (v/v) were prepared as described above and sealed with thick rubber stoppers and aluminum crimps. The incubation solution was

either unamended with any of electron acceptors as the "anoxic unamended" treatment (as measured in the unamended incubation solution: NO<sub>3</sub><sup>-</sup>: 0.03 mM; SO<sub>4</sub><sup>2-</sup>: 0.31 mM), or amended with either NaNO<sub>3</sub> (20 mM), Na<sub>2</sub>SO<sub>4</sub> (20 mM), or Fe (III) citrate (20 mM), as an electron acceptor to stimulate nitrate-reducing, sulfate-reducing, or Fe (III)-reducing conditions. Sodium sulfide (1 mM) and Na-lactate (10 mM) were added to all three-reducing treatments, serving as reducing agent and electron donor, respectively [24]. No measurements of redox potentials (Eh) were made to confirm the reducing condition of the treatment because relatively high concentrations of the electron acceptors (nitrate, sulfate, and Fe [III]) have been used to achieve effective reducing conditions. Based on the measurement of nitrate and sulfate concentrations in the nitrate- and sulfate-reducing treatments during the incubation, we could know whether the nitrate-reducing and sulfate-reducing conditions in the treatments were achieved effectively. To maintain anoxic conditions, the anaerobic chamber was flushed with a mixture of N<sub>2</sub>/CO<sub>2</sub> gas (80:20, v/v). All the treatments contained the redox indicator resazurin at 0.0002% (w/w). The medium changed from pink to colorless when the anoxic condition in each bottle was reached. Nitrate-amended samples had to be supplemented further with nitrate (20 mM) to maintain the nitrate-reducing condition after complete depletion during the period of incubation. At each sampling point, the cultures were shaken vigorously and sampled with sterile syringes. All bottles were incubated in the dark at 25°C inside the anaerobic chamber.

### Extraction and analysis

**Analysis of BP-3.** For analysis of residual concentrations of BP-3, a 15-ml aliquot of slurry was withdrawn from each treatment using a glass syringe and placed into glass culture tubes. The tubes were then screw-capped using PTFE lined caps and centrifuged at 800g for 20 min (Ananti30, Bechman). The resulting supernatant was extracted/cleaned by using solid phase extraction cartridges (Oasis HLB 6 ml, 500 mg; Waters). The remaining pellets in the tubes were then extracted three times by ultrasonication (Branson Ultrasonics, 5510E-DTH) with 2 ml methanol for 10 min, and after centrifugation, the supernatant was diluted with Milli-Q water (10 ml) and put through the same solid phase extraction cartridge with the previous supernatant. Solid phase extraction cartridges were preconditioned with 2 × 3 ml dichloromethane, 2 × 3 ml methanol, and 2 × 3 ml Milli-Q water in sequence before loading the samples. The cartridges were dried under vacuum for 3 h and the target analytes were eluted from the cartridges with 3 × 2 ml of dichloromethane/methanol (50:50 v/v). The extracts were evaporated to dryness under a gentle stream of nitrogen and then reconstituted in 950 µl of acetone and 50 µl of 2 mg/L benzylcinnamate internal standard was spiked into each sample to obtain a final concentration of at 100 µg/L. Each of the final extracts were then filtered through a nylon syringe filter (13 mm × 0.22 µm, Anpu) into a 2-ml amber glass vial for analysis.

All extracts were analyzed by gas chromatography-tandem mass spectrometry (GC-MS/MS, Agilent 7000A/7890A). Target compound in the samples was separated on a HP-5MS column (30 m × 0.22 mm, 0.25 µm thickness) with helium as carrier gas at a linear flow rate of 1.656 ml/min. The GC oven temperature was programmed from 80°C (hold 2 min) to 280°C (hold 6 min) at a rate of 15°C/min. The injection port temperature was 280°C and transfer line temperature was 280°C. The MS/MS was operated in multiple reaction monitoring mode.

Ionization was carried out in high sensitivity electron impact mode at 70 eV, with the ion source temperature at 230°C. The multiple reaction monitoring details of the target compound and internal standard are shown in Supplemental Data, Table S1. The target compound was identified by comparing the retention times (within 2%) and the ratios (within 20%) of the two selected precursor-product ion transitions with the standard of BP-3. Quantification was performed using the internal standard method. Laboratory blanks were also analyzed along with the samples to assess potential sample contamination. Data acquisition was performed under Agilent Mass Hunter (Ver B.03.01) application.

Recovery experiments were performed by spiking the standard solution of BP-3 (1 mg/L) to three parallel sterile incubation solutions (10% of inocula, v/v) and processed using the above-described sample extraction methods. The recovery by using the above extraction and analysis method was 121% for BP-3.

**Analysis of biodegradation products for BP-3.** The biodegradation products of BP-3 under different conditions (oxic, nitrate-reducing, sulfate-reducing, Fe [III]-reducing, and anoxic unamended) after 42 d of incubation were extracted (80 ml of slurry sample each) by using a solid phase extraction as described earlier and analyzed using an Agilent 6890 gas chromatography coupled to a 5973 mass spectrometric detector (GC-MS) and a Thermo Finnigan TSQ Quantum liquid chromatography-MS/MS (LC-MS/MS). Detailed instrumental conditions are given in the Supplemental Data. The mass spectrum of each peak in the total ion chromatograms of GC-MS in scan mode was deconvoluted and peaks were assigned identities using an automated mass spectral deconvolution and identification system (National Institute of Standards and Technology), which is able to identify chemical structures, estimate molecular weight, and generate chemical formulas for compounds corresponding to the respective peaks [25,26].

**Analysis of nitrate, sulfate, and bacterial counting.** To monitor the electron acceptors in the medium, nitrate and sulfate in the cultures were measured using a Dionex ICS-2500 ion chromatograph (Dionex), equipped with a 2 mm AS16 anion separation column and hydroxide eluent generated on-line followed by conductivity detection after chemical suppression. Total numbers of culturable bacteria in the media of each treatment were monitored on each sampling occasion using the most probable number technique (Supplemental Data) [27].

#### Statistical analysis

All statistical analyses were performed with statistical product and service solutions (SPSS 13.0). Data of BP-3

Table 1. Kinetic parameters for the biodegradation of benzophenone-3 (BP-3) under oxic, anoxic unamended, nitrate-reducing, sulfate-reducing, and Fe (III) reducing conditions

Redox condition	$k$ (1/d) <sup>a</sup>	$r^2$ <sup>b</sup>	$t_{1/2}$ (d) <sup>c</sup>
Oxic	$0.0647 \pm 0.0033$	0.941	$10.7 \pm 0.54$
Anoxic unamended	$0.1631 \pm 0.0028$	0.913	$4.2 \pm 0.07$
Nitrate reducing	$0.0800 \pm 0.0007$	0.934	$8.7 \pm 0.08$
Sulfate reducing	$0.1605 \pm 0.0006$	0.930	$4.3 \pm 0.02$
Fe (III) reducing	$0.1366 \pm 0.0028$	0.847	$5.1 \pm 0.11$

<sup>a</sup> Kinetic rate constant, which was predicted by using the first-order reaction kinetic model (mean values from two replicate experiments were used in the calculation).

<sup>b</sup> Correlation coefficient, which represents the fitness of the modeling data.

<sup>c</sup> Half-life, which is calculated as  $0.693/k$ .

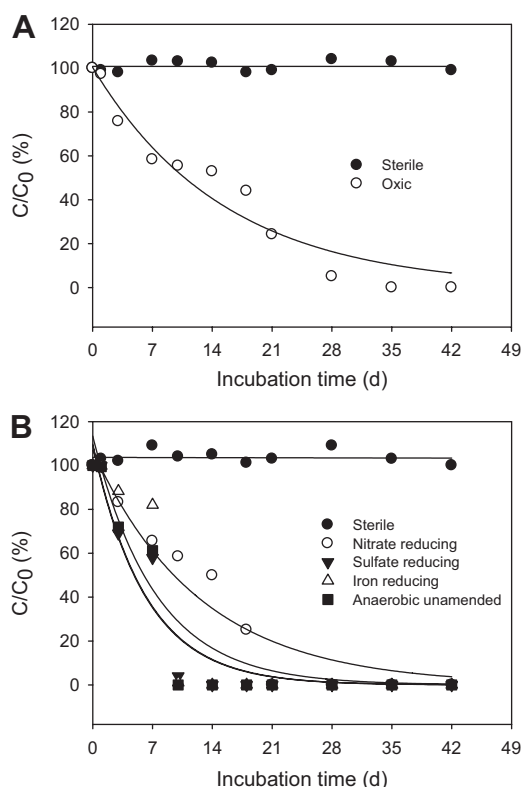


Fig. 1. Aerobic (A) and anaerobic (B) biodegradation of benzophenone-3 (BP-3; initial concentration of 1 mg/L) by using 10% of activated sludge and digested sludge as inocula, respectively.

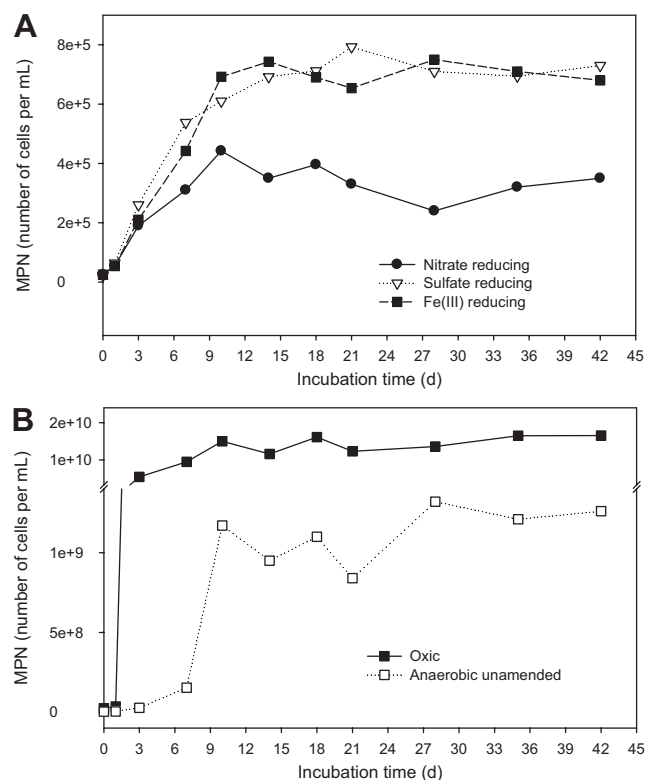


Fig. 2. Total numbers of culturable bacteria in nitrate-reducing, sulfate-reducing, and Fe (III)-reducing microcosms (A) and in oxic and anoxic unamended microcosms (B) of benzophenone-3 (BP-3) biodegradation experiments during incubation period.

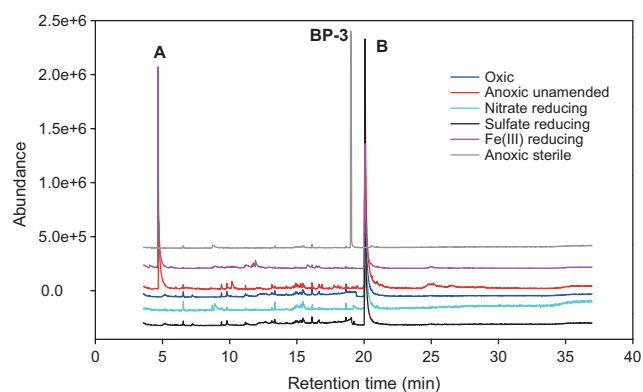


Fig. 3. Total ion chromatograms of gas chromatography-mass spectrometry (GC-MS) of benzophenone-3 (BP-3) under different redox conditions after 42 d incubation. (A) 4-cresol; (B) 2,4-dihydroxybenzophenone.

biodegradation half-lives from different treatments were analyzed by one-way analysis of variance. Mean separations were performed by Duncan's multiple range tests. Differences at  $p < 0.05$  were considered significant.

## RESULTS AND DISCUSSION

### Biodegradation of BP-3 under different redox conditions

Benzophenone-3 was found to be stable under sterile conditions. No hydrolysis occurred and any removal caused by volatilization during testing could also be accounted for based on the data for the sterile controls in each treatment. The biodegradation kinetics of BP-3 followed the first-order model (Supplemental Data) and the corresponding kinetic parameters including kinetic rate constant ( $k$ ) and half-life ( $t_{1/2}$ ) are summarized in Table 1. Significant differences in biodegradation half-lives were observed between different treatments studied except for that between sulfate-reducing and anoxic unamended conditions.

Benzophenone-3 was completely biodegraded in both aerobic and anaerobic microcosms within 42 d of incubation (Fig. 1,

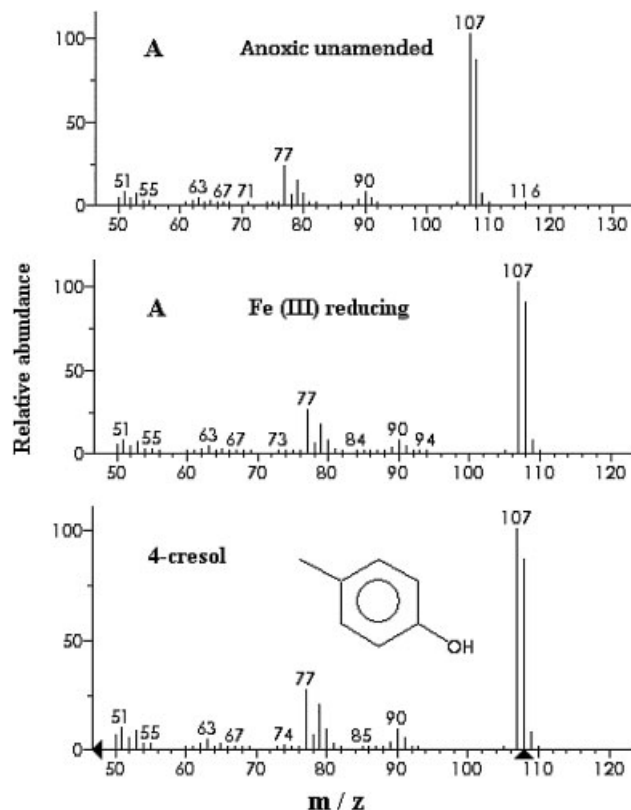


Fig. 4. Mass spectra of 4-cresol standard and corresponding metabolite A produced from benzophenone-3 (BP-3) degradation under anoxic unamended and Fe (III)-reducing conditions.

Table 1). Differences in biodegradation half-lives for BP-3 were observed under the following different redox conditions: oxic > nitrate-reducing > Fe (III)-reducing > sulfate-reducing and anoxic unamended. The relatively short half-lives of BP-3 (4.2–10.7 d) in oxic and anoxic conditions suggested that BP-3 was easily biodegraded and would be efficiently removed from the sewage treatment system within normal hydraulic retention

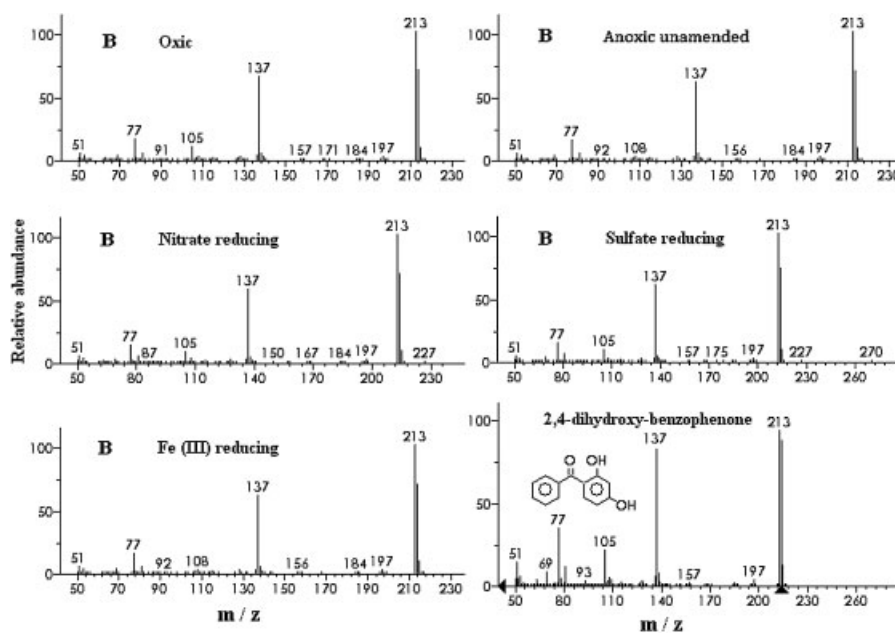
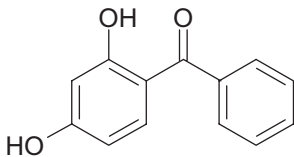

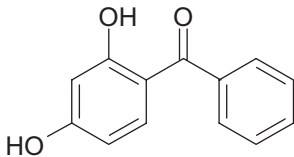
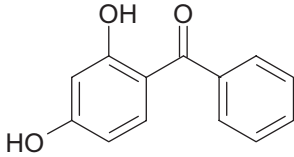
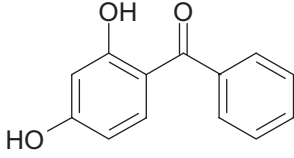
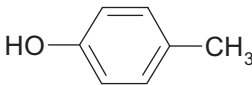
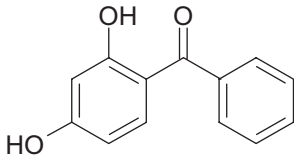


Fig. 5. Mass spectra of 2,4-dihydroxybenzophenone standard and corresponding metabolite B produced from benzophenone-3 (BP-3) degradation under oxic, anoxic unamended, nitrate-reducing, sulfate-reducing, and Fe (III)-reducing conditions.

Table 2. Tentative structures and relevant data of the benzophenone-3 (BP-3) biodegradation products

AMDIS program and NIST05 library results									
	Peak	Retention time (min)	RI	Molecular weight	Empirical formula	RI	Structure	Name	Match
Oxic	B	20.02	2659	214	C <sub>13</sub> H <sub>10</sub> O <sub>3</sub>	2644		2,4-dihydroxy benzophenone	916
Anoxic unamended	A	4.71	1109	108	C <sub>7</sub> H <sub>8</sub> O	1055		4-cresol	964
	B	20.02	2659	214	C <sub>13</sub> H <sub>10</sub> O <sub>3</sub>	2644		2,4-dihydroxy benzophenone	900
Nitrate reducing	B	20.02	2659	214	C <sub>13</sub> H <sub>10</sub> O <sub>3</sub>	2644		2,4-dihydroxy benzophenone	913
Sulfate Reducing	B	20.02	2659	214	C <sub>13</sub> H <sub>10</sub> O <sub>3</sub>	2644		2,4-dihydroxy benzophenone	942
Iron reducing	A	4.71	1109	108	C <sub>7</sub> H <sub>8</sub> O	1055		4-cresol	966
	B	20.02	2659	214	C <sub>13</sub> H <sub>10</sub> O <sub>3</sub>	2644		2,4-dihydroxy benzophenone	936

AMDIS = Automated Mass Spectral Deconvolution and Identification System; NIST05 = Version 05 of mass spectral library provided by National Institute of Standards and Technology (Gaithersburg, MD, USA); RI = retention index, calculated by using AMDIS and NIST mass spectral search program 2.0 in the evaluation of the library hits and experiment results.

times. This is consistent with the removal efficiencies (68–96%) in conventional WWTPs [2,6]. The half-lives for BP-3 under oxic conditions are much longer than those in anoxic conditions, indicating that anoxic conditions are more favorable to BP-3 biodegradation than are oxic conditions. Inhibition of anaerobic degradation of organic compounds in the presence of nitrate and sulfate is well documented in the literature [20,28,29]. Biodegradation of BP-3 was also observed under Fe (III)-reducing conditions. Iron (III) reduction is a common anaerobic terminal electron-accepting process in anaerobic systems of the natural environment [30,31]. This suggests that BP-3 in the natural environment would be less persistent under Fe (III)-reducing condition than under oxic conditions.

Nitrate and sulfate, as well as microbial activity, were monitored during the incubation period. Anoxic NaNO<sub>3</sub> or Na<sub>2</sub>SO<sub>4</sub> solution was added to either the NO<sub>3</sub><sup>-</sup> amended treatments or SO<sub>4</sub><sup>2-</sup> amended treatment to yield initial dissolved NO<sub>3</sub><sup>-</sup> concentrations of 16.32 mM or SO<sub>4</sub><sup>2-</sup> concentrations of 19.75 mM, respectively. Nitrate reduction during the degradation of BP-3 followed a time course with initial rapid nitrate depletion (first 3 d) followed by a moderate decrease in nitrate concentration after the re-amendment of nitrate during the remaining incubation period in the nitrate-reducing treatments (Supplemental Data, Fig. S1). The depletion rate of sulfate in BP-3 treatment under sulfate-reducing condition showed a slow decrease in sulfate concentration during the incubation period (Supplemental Data, Fig. S2).

Microbial activity in the treatments for BP-3 rapidly increased from day 0 to day 10 for all redox conditions (Fig. 2). These changes in microbial number seem to correlate positively with the biodegradation of BP-3, because the removal rate of BP-3 had obviously increased during this stage under each redox condition (Fig. 1). After the initial increasing phase, the microbes maintained stable growth. The total numbers of culturable aerobic bacteria ( $2.4 \times 10^7$  to  $1.65 \times 10^{10}$ ) were found much higher than those of anaerobic bacteria ( $3.0 \times 10^6$  to  $1.3 \times 10^9$ ).

#### Biodegradation products of BP-3

Two metabolites, including 4-cresol and 2,4-dihydroxybenzophenone, were detected as the products of BP-3 under anoxic unamended condition and Fe (III)-reducing conditions, while only 2,4-dihydroxybenzophenone was detected under oxic, nitrate-reducing, and sulfate-reducing conditions (Fig. 3). Both metabolites were identified by using automated mass spectral deconvolution, and identification system, and by comparison of their mass spectra to the authentic compound (Figs. 4, 5; Table 2). Biodegradation products of BP-3 under each redox condition were also verified by LC-MS/MS analysis of the samples (Supplemental Data, Fig. S3). One main peak at  $m/z$  215 was detected and identified as 2,4-dihydroxybenzophenone in each redox condition.

It should be noted that the biodegradation products of BP-3 after 42 d of incubation under various redox conditions were determined only for a single timepoint (end of the incubation) and were tentatively identified only based on GC-MS and LC-MS/MS results by using automated mass spectral deconvolution and the identification system program and NIST05 database. Future studies should include more sampling time-points for product identification during the incubation period to monitor the transformation of both parent and product compounds. Identification of degradation products should be further confirmed by using synthetic standards, time-of-flight MS, and nuclear magnetic resonance.

#### Proposed degradation pathways of BP-3

Based on the identified products, biotransformation pathways of BP-3 under each redox condition have been tentatively proposed (Fig. 6). In the present study, 2,4-dihydroxybenzophenone was detected in aerobic biotransformation of BP-3. We propose that transformation of BP-3 to 2,4-dihydroxybenzophenone by way of demethylation of the methoxy substituent (*O*-demethylation) occurred in cultures under oxic condition. The initial *O*-demethylation of BP-3 to 2,4-dihydroxybenzophenone was also detected under all four anoxic conditions (anoxic unamended, nitrate-, sulfate-, and Fe [III]-reducing conditions) in the present study. *O*-demethylation of organic compounds has previously been demonstrated under oxic and anoxic conditions [32,33]. Biodegradation of dicamba through the *O*-demethylated product 3,6-dichlorosalicylate under oxic and anoxic conditions (methanogenic, denitrifying, and sulfate-reducing) is well documented [20,32,34]. Under anoxic conditions, the relatively longer half-life of BP-3 under the nitrate-reducing condition indicated that *O*-demethylation was inhibited by nitrate. Milligan and Haggblom [20] demonstrated an inhibition of *O*-demethylation in the presence of nitrate, while *O*-demethylation occurred at faster rates and to a greater extent in the enrichments once nitrate concentrations were reduced below 4 mM. 4-cresol was identified as the biodegradation product of BP-3 under anoxic unamended condition and Fe (III)-reducing conditions, which could be the secondary byprod-

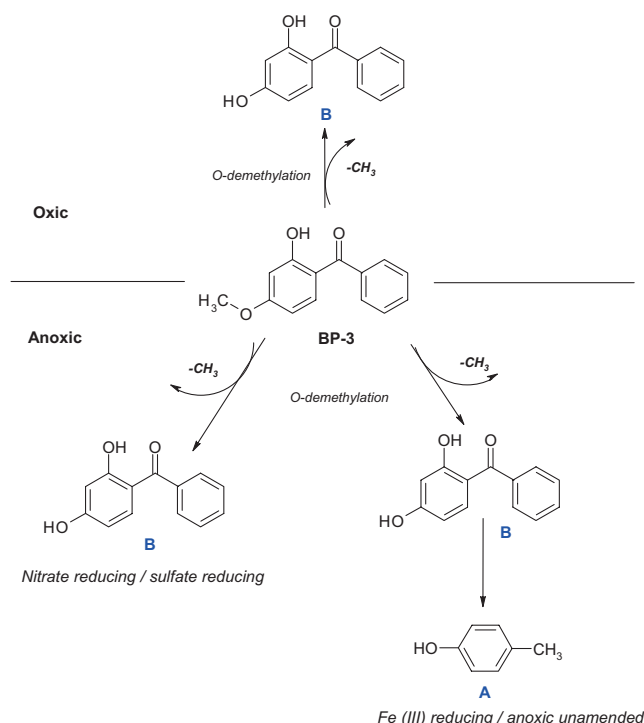


Fig. 6. Proposed schemes for the biodegradation of benzophenone-3 (BP-3) under different redox conditions. The biodegradation products of BP-3 were tentatively identified by GC-MS and liquid chromatography-tandem mass spectrometry (LC-MS/MS) and further confirmed by using the Automated Mass Spectral Deconvolution and Identification System (AMDIS) and the National Institute of Standards and Technology (NIST 05) database searching program. (A) 4-cresol; (B) 2,4-dihydroxybenzophenone.

uct by further transformation of 2,4-dihydroxybenzophenone. The University of Minnesota Biocatalysis/Biodegradation Database ([35]; <http://unbbd.ahc.umn.edu/>) was also applied here to predict potential biotransformation products and pathways of 2,4-dihydroxybenzophenone, but the modeling did not find the metabolite 4-cresol, which shows that the metabolite 4-cresol may be an artifact. Further investigation is needed to explore the biotransformation of BP-3 under various redox conditions.

#### CONCLUSION

Based on the results from the present study, it can be concluded that BP-3 is positively biodegradable under each redox condition. The redox conditions had significant influences on the biodegradation rates of BP-3. *O*-demethylation of BP-3 leading to the formation of 2,4-dihydroxybenzophenone was tentatively proposed as the main pathway for the biodegradation of BP-3 under each redox condition. Specific redox conditions can be created by the amendment of certain electron acceptors to improve biodegradation of BP-3. Anoxic conditions are more favorable for BP-3 to be degraded biologically. These findings could have significant implications for the in situ biodegradation of BP-3 in oxic and anoxic water, soil, and sediments.

#### SUPPLEMENTAL DATA

The Supplemental Data includes one table and three figures. (1.0 MB DOC).



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