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BIODEGRADATION OF BISPHENOL A IN AQUATIC ENVIRONMENTS: RIVER DIE-AWAY

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Abstract—The biodegradability of bisphenol A (BPA) was assessed in surface waters from seven different rivers across the United States and Europe. Rapid biodegradation of BPA was observed in all rivers following lag phases ranging from 2 to 4 d. Biodegradation half-lives for BPA were typically less than 2 d following the lag phase. Mineralization of BPA was observed in all river waters, with average carbon dioxide yields of approximately 76% of the theoretical maximum (range 59–103%) at the end of the incubation period (≤18 d). Short half-lives (0.5 to 3 d) were noted for BPA biodegradation in river waters regardless of geographic location, sampling site (i.e., upstream vs downstream of wastewater outfalls), sediment addition (≤0.05%), and initial test chemical concentration (50–5,500 μg/L). Subsequent studies conducted at environmentally relevant concentrations (0.05 and 0.5 μg/L) also indicated short half-lives (3–6 d) for BPA and support the extrapolation of the half-lives measured in this study over a wide range of environmental concentrations. The fact that BPA was degraded rapidly in surface waters taken from diverse locations in the United States and Europe as well as in studies recently conducted in Japan suggests that BPA degrading microorganisms are widely distributed in nature. These observations provide clear evidence that BPA is not persistent in the aquatic environment.

Keywords—Biodegradation Bisphenol A River die-away Kinetics Half-life

INTRODUCTION

Bisphenol A (BPA; 2,2-(4,4-dihydroxydiphenyl)propane; CAS 80-05-7) is a large-volume chemical intermediate used in the production of polycarbonate and epoxy resins, flame-retardants, and other specialty products. The BPA may be released into the environment via permitted outfalls of industrial or public-owned wastewater facilities that receive BPA. The physical properties of BPA indicate that the compound will tend to remain in the aqueous environment [1]. Based on a water solubility of 120 mg/L, a vapor pressure of 4.0×10^{-8} mm Hg, and the molecular weight (228 g/mol), a Henry's constant (H) of 1.0×10^{-10} atm-m^3/mol has been calculated [2]. The magnitude of this value (less than 1.0×10^{-7} atm-m^3/mol) indicates that the compound will have little tendency to volatilize from water [2]. Measured soil adsorption coefficient (K_{oc}) values for BPA have not been reported, but values estimated from water solubility and octanol/water partition coefficient measurements range from 314 to 1,524 [2]. The magnitude of these values indicates that some adsorption of BPA to soil and sediments would be expected. In addition, predictions of the distribution of BPA in the environment based on level 1 fugacity modeling (degradation processes excluded) suggest that approximately half of the BPA would be associated with soil or sediments [1].

Biodegradation is expected to be the dominant process for removal of BPA from the aquatic environment. The biodegradability of BPA has been examined in a variety of laboratory test systems using both acclimated and nonacclimated microorganisms. These studies have been recently reviewed by Staples et al. [1]. The compound has been shown to meet the criteria for both ready and inherent biodegradability in laboratory studies conducted according to current Organisation for Economic Cooperation and Development (OECD) guidelines [3,4]. Biological treatment of wastewater containing BPA has also been simulated in laboratory studies; results of semicontinuous activated sludge tests showed 87 to 95% degradation [5]. In addition, biodegradation pathways have been characterized using a strain of gram-negative bacteria isolated from an industrial wastewater treatment plant [6,7]. Treatment of wastewaters containing BPA has been examined in industrial wastewater treatment plants, with reduction of BPA concentrations ranging from 92 to 99% in separate studies [1,8,9].

Results of a previous river die-away study suggest that BPA will not persist in surface waters. Dorn et al. [10] examined biodegradability of 3,000 μg/L BPA in water samples collected from the effluent of a manufacturing facility, the receiving stream, and the Houston Ship Channel (Houston, TX, USA) and reported half-lives in the range of 2.5 to 4 d. Although the available data suggest that BPA would be expected to degrade rapidly in receiving waters downstream from manufacturing facilities, there are only limited data to support this conclusion. The purpose of this study was to assess the aerobic biodegradability of BPA under conditions that simulate a wide variety of surface-water environments. The objectives of the study were to determine the range of half-lives for BPA biodegradation that might be expected in surface waters, evaluate the effects of preexposure and adaptation on biodegradation kinetics by examining the biodegradation of BPA in samples taken upstream and downstream of outfalls of wastewater treatment systems known to receive BPA, investigate the role of sediments in determining the fate of BPA in the aquatic environment, and determine the potential for and kinetics of BPA biodegradation at environmentally relevant (nanogram per liter) concentrations.

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MATERIALS AND METHODS

Test substance

The BPA was obtained from Research Triangle Institute (Research Triangle Park, NC, USA). The sample was identified by lot number B0070138. The purity was reported to be 99.1%, and the supplier confirmed the identity by infrared spectroscopy and nuclear magnetic resonance spectroscopy (^{1}H-NMR).

Radiolabeled [^{14}C]BPA (uniformly ring labeled) was obtained from Wizard Laboratories (West Sacramento, CA, USA). The sample was identified by lot number 980622. Radiochemical purity was reported to be 99.5%, with a specific activity of 41.17 milliCuries/mmole. Upon receipt, the compound identity was confirmed by gas chromatography with electron impact mass spectroscopy. The radiochemical purity and specific activity specified by Wizard Laboratories were confirmed by high-performance liquid chromatography (HPLC) with radiochemical and ultraviolet (UV) detection.

Chemicals used as analytical reference compounds, reagents, and solvents were obtained from commercial sources with the appropriate documentation of purity. Deionized water was purified in a Milli-Q Water Purification System (Millipore®, New Bedford, MA, USA).

River water and sediment samples

River water and sediment samples were collected from eight locations on seven rivers or estuaries across the United States and Europe from July 1998 to January 2000. Samples were collected both upstream and downstream of outfalls of wastewater treatment systems known to receive BPA. Sampling locations included the Ohio River in Mt. Vernon, Indiana, USA; the Ware River in Ware, Massachusetts, USA; the Monte Sano Bayou in Baton Rouge, Louisiana, USA; the Mississippi River in Baton Rouge, Louisiana, USA; the Rhine River in Krefeld, Germany; the Elbe River in Stade, Germany; and the Westerschelde River in Bergen-op-Zoom, The Netherlands.

The procedure for collecting water and sediment samples depended on the depth and size of the river. For the Rhine, Ohio, and Westerschelde rivers, water sampling required use of a boat. For the other rivers, water samples were collected from the bank or by wading into the river. Water samples were collected by immersing 4-L amber glass bottles just below the surface to minimize collection of floating debris. During sampling, a number of water quality parameters were measured, including water temperature, pH, dissolved oxygen concentration, and conductivity.

Surface sediment samples (i.e., the top 1–2 cm) were collected from depositional zones in 1-L polyethylene bottles. A diver was used to locate and collect sediments from the Ohio River. Sediments from the Rhine and Westerschelde rivers were collected using grab sampling devices operated from boats. For the remaining rivers, sediment samples were collected from the bank or by wading into the river and scooping the sediment into the collection bottle.

Water and sediment samples were chilled with ice packs and shipped via overnight express to the laboratory. Upon receipt, separate composite samples of upstream and downstream sediment samples were prepared and passed through a 2-mm sieve to remove stones and improve homogeneity. Water samples containing high levels of particulates (by visual inspection) were filtered through glass wool prior to use. Die-away studies were set as soon as possible, generally within 24 to 48 h of receipt of the samples.

Subsamples of the composite sediment and water samples were submitted for characterization to Midwest Laboratories (Omaha, NE, USA). Sediment samples were analyzed for texture, organic carbon content, inorganic content, cation exchange capacity, and total heterotrophic bacteria. River water samples were analyzed for conductivity, alkalinity, anions, cations, total dissolved solids, and total heterotrophic bacteria.

Experimental approach

The experimental design for the river die-away studies was based on standard methods issued by the U.S. Environmental Protection Agency (U.S. EPA), the American Society for Testing and Materials, and the Society of Environmental Toxicology and Chemistry [11–13]. The effects of BPA concentration, river sediment concentration, and microbial adaptation on BPA degradation kinetics were addressed in the experimental design. The degradation of [^{14}C]BPA was routinely followed through at least three half-lives or greater than 90% degradation. The duration of the studies was sufficient to define the degradation pattern of BPA and possible metabolites. All phases of the study were conducted in compliance with Good Laboratory Practice Standards as described by the U.S. EPA [14] and OECD [15].

The biodegradation of BPA was examined in river die-away experiments using both surface water alone and sediment/water mixtures. Initial biodegradation experiments were run using two different methods conducted in parallel. With one method, [^{14}C]BPA was added to river water and water/sediment mixtures in sealed shake flasks fitted with CO_2 traps and sampling ports. The advantage of this procedure was the ability to examine [^{14}C]BPA biodegradation over a range of initial test chemical concentrations and to monitor the fate the chemical, formation of [^{14}C]metabolites, and the mineralization of [^{14}C]BPA to $^{14}CO_2$. Studies were also performed using a Columbus MicroOxymax respirometer (Columbus Instruments, Columbus, OH, USA), where oxygen consumption and production of CO_2 due to the biodegradation of BPA were measured. The advantages of the respirometer were efficiency, continuous monitoring of the degradation process, and low cost, although higher concentrations of BPA (~5,000 μg/L) were required to achieve the necessary sensitivity.

Biologically inhibited (killed) controls were included in all studies to verify degradation was due to biological activity. Killed controls were prepared by autoclaving the water and sediment (121°C, 15 psi, 30 min) prior to the addition of BPA. Autoclaving was used because preliminary experiments indicated that chemical sterilants such as mercuric chloride (500 mg/L) or formalin (2%) could react with BPA under the test conditions.

[^{14}C]BPA die-away studies

Initial die-away experiments were conducted with [^{14}C]BPA to define biodegradation kinetics over a range of BPA concentrations. The range was bounded at the low end by the detection limit of the analytical methods and at the high end by the lowest concentration that could be tested in the respirometer (5,000 μg/L). Microcosms mixtures were prepared by placing 200-ml portions of river water in 500-ml test vessels. Reaction mixtures were amended with 50 and 500 μg/L [^{14}C]BPA by the addition of 15 μl portions of stock solutions of [^{14}C]BPA in 1,4-dioxane. To prepare microcosms containing 5,500 μg/L [^{14}C]BPA, a large volume of water (5 L) was initially amended with 25,000 μg of nonlabeled BPA, distrib-

uted into the test vessels, and then supplemented with an additional 500 μg/L of [^{14}C]BPA. Final BPA concentrations in the amended river waters were confirmed by HPLC analyses. Following the addition of [^{14}C]BPA to the reaction mixtures, the headspace gases of the microcosms were sparged with oxygen (to ensure that aerobic conditions were maintained) and sealed with rubber stoppers. The stoppers were fitted with CO_2 traps containing 4 ml of 1 N NaOH, which were suspended in the flask headspace, and a sampling port to permit sampling of the reaction mixtures. All microcosms were incubated at 20 ± 2°C in the dark. To ensure maintenance of aerobic conditions, the microcosms were mixed on a rotary shaker at 100 rpm.

At various time intervals, 2-ml samples were removed from duplicate microcosms, mixed with 0.5 ml of acetonitrile for 30 min, and then filtered through 0.7-μm glass-fiber filters. Recovery of [^{14}C]BPA from river water alone and from water/sediment mixtures using this procedure was >98%. The filtrates were analyzed for total radioactivity by liquid scintillation counting, and the distribution of radioactivity between [^{14}C]BPA and [^{14}C]metabolites was determined by HPLC. At various time intervals, the contents of the CO_2 traps were replaced with fresh 1 N NaOH. The amount of $^{14}CO_2$ collected in the traps was quantified by liquid scintillation counting (LSC).

To investigate the effects of high sediment concentrations on the biodegradation of BPA, reaction mixtures containing 10% weight/weight (w/w) sediment were prepared for downstream samples from the Rhine River. Sediment samples (2.0 g dry wt) were combined with 18 ml of river water in 100-ml serum bottles, amended with 500 μg/L [^{14}C]BPA, sealed, and incubated as described above. At various time intervals, selected microcosms were mixed with 5-ml portions of acetonitrile for at least 30 min, and the supernatant liquids were filtered and analyzed by LSC and HPLC as described below to measure the concentrations of [^{14}C]BPA and [^{14}C]metabolites present. Separate microcosms were acidified with concentrated phosphoric acid (0.5 ml) and sparged with nitrogen, which was passed through two caustic traps (1 N NaOH) arranged in series. The traps were combined and the amount of radioactivity collected as [^{14}C]carbonate was measured using LSC. At the conclusion of the experiment, sediments were combusted to measure the amount of radioactivity bound to the sediment or incorporated into biomass.

Respirometer studies

The BPA biodegradation in the respirometer was examined at a test chemical concentration of 5,000 μg/L. This concentration represented the lowest level for which biodegradation could be accurately measured, based on the sensitivity of the instrumentation. Five-liter portions of either upstream or downstream river water were amended with BPA at a nominal concentration of 5,000 μg/L as described above. Reaction mixtures were prepared in duplicate in closed 1-L reaction vessels, each containing 500-ml portions of river water. Blank controls (without added BPA) were also prepared in duplicate and were used to determine endogenous O_2 consumption and CO_2 production by the indigenous microorganisms. The test mixtures were incubated at a temperature of 20 ± 2°C in the dark and continuously stirred with a magnetic stir bar at 100 rpm. Measurements of gas-phase O_2 and CO_2 in the reaction vessels occurred at 6-h intervals during the test. Measurements of pH, nitrite, and nitrate concentrations were performed at the beginning and the conclusion of the experiment using standard procedures. Nitrite and nitrate measurements were used to confirm that the oxygen consumption observed in the respirometer was due to the biodegradation of BPA and not due to the oxidation of ammonia that may have been present in the reaction mixtures.

In addition to reaction mixtures prepared with water alone, microcosms were also prepared with river water and 0.05% sediment (dry wt) using both upstream and downstream samples. Composite sediment samples (250 mg) were added to 500-ml portions of river water amended with 5,000 μg/L BPA. Corresponding blank mixtures (no BPA added) were also prepared. Higher sediment concentrations were not run in the respirometer due to an anticipated excessive oxygen demand.

Low-level die-away study

Additional studies were conducted to determine whether the kinetics of BPA biodegradation determined for test chemical concentrations of greater than 50 μg/L could be extrapolated to predict the fate of the compound at the low nanogram-per-liter levels reported in the environment [16,17]. Surface water samples for the study were collected from the Mississippi River. Microcosms were prepared by dispensing 500-ml portions of river water into separate 1-L glass bottles. The BPA was added at nominal concentrations of 0.05 and 0.5 μg/L to two sets of microcosms; the test chemical was added as an aqueous stock solution. The third group of microcosms was not amended to determine the fate of potential background concentrations of BPA. All microcosms were incubated with mixing at 20 ± 2°C in the dark.

Separate microcosms were analyzed on days 0, 7, 18, and 28. In general, triplicate viable microcosms and single or duplicate killed controls were analyzed on each sampling day. Prior to sample work-up, the microcosms were amended with D_8-BPA (Cambridge Isotope, Andover, MA, USA) as an internal standard (~1 μg/L) to improve the accuracy and precision of the analysis. Microcosms also received 100 μl hydrochloric acid and 15 g of sodium chloride to acidify and increase the ionic strength of the river water to improve the recovery of BPA during solvent extraction. The bottles were mixed by shaking, and the contents were transferred to a separatory funnel. The samples were extracted with three separate 15-ml portions of methylene chloride, and the combined extracts were collected over 20 g of Na_2SO_4. The extracts were reduced to dryness using vacuum centrifugation at room temperature. The residue was derivatized with 200 μl trifluoroacetic acid (TFA) at room temperature for 15 min. The excess reagent was evaporated to dryness under a gentle stream of N_2 and the BPA-TFA derivative was reconstituted in 250 μl of toluene and quantified by gas chromatography/electron impact ionization/mass spectroscopy (GC/EI/MS) as described below. Matrix calibration standards consisting of 500-ml aliquots of Milli-Q water fortified with known levels of BPA (~0.005–0.5 μg/L) and the same level of D_8-BPA internal standard were prepared in the same manner as the samples.

Analytical methods

A reverse phase HPLC system with UV detection and radioactivity monitoring (RAM) was initially used to measure background BPA concentrations in the river water samples, confirm the identity and radiochemical purity of the [^{14}C]BPA test chemical, and measure changes in concentrations of [^{14}C]BPA and [^{14}C]metabolites in reaction mixtures. A mobile

phase containing 34% (v/v) acetonitrile and 1% acetic acid in water was delivered at 1.0 ml/min with a Hitachi L6200A HPLC pump (Tokyo, Japan). Samples (75–200 μl) were injected onto the column using an Alcott 738 autosampler. A ZORBAX SB-C8 column (4.6 × 150 mm, MAC-MOD Analytical, Chadds Ford, PA, USA) was used to separate [^{14}C]BPA from [^{14}C]metabolites. The [^{14}C]BPA eluted at approximately 14 min under the conditions cited above. Compounds in the column effluent were detected with a SpectroMonitor D UV detector (LDC Analytical, Riviera Beach, FL, USA) at 254 nm in series with a Berthold model LB 506 C-1 HPLC Radioactivity Monitor (EG&G, Burlington, MA, USA) equipped with a GT-400 solid-phase cell. The output from both detectors was connected to a PE Nelson data system (Cupertino, CA, USA). The approximate detection limit (three times signal to noise ratio; UV detection) for measurement of background BPA concentrations in the river water samples ranged from 50 to 100 μg/L, depending on the volume of sample injected. The approximate detection limit (three times signal/noise ratio; radiochemical detection) for the measurement of [^{14}C]BPA in the reaction mixtures was 7 μg/L.

Total radioactivity in test samples was determined by liquid scintillation counting using an LS-6002 liquid scintillation counter (Beckman Instruments, Fullerton, CA, USA). Liquid samples (200 μl) were mixed with 6 ml Aquasol liquid scintillation cocktail (NEN Life Sciences, Boston, MA, USA). For caustic samples from the CO_2 traps, 200-μl portions were mixed with 500 μl of water and 5 ml of Aquasol. The identity of $^{14}CO_2$ in the solutions was confirmed by precipitation with barium nitrate [18]. Standard solutions for radioactivity counting were prepared with [^{14}C]toluene from NEN Life Sciences. Radioactivity measurements were corrected for quenching and counting efficiency.

Total radioactivity bound in the sediment samples was determined by combustion in a Biological Oxidizer model OX-300 (R.J. Harvey Instrument, Hillsdale, NJ, USA). Sediment samples were air dried and triplicate 0.5-g portions were typically combusted in the oxidizer. The exhaust gases were passed through 15 ml of Carbon-14 Cocktail (R.J. Harvey) to trap $^{14}CO_2$ produced from the combustion of the radioactive residues contained in the sediment.

Biodegradation of BPA in the respirometer was determined by measuring O_2 consumption and CO_2 production in reaction mixtures over time. Oxygen consumption and CO_2 production due to degradation of BPA was determined by subtracting background values measured in the blank controls. The system used a paramagnetic O_2 sensor with a measurement range of 19.0 to 21.0% (vol) O_2 and a nondispersed infrared CO_2 detector with a measurement range of 0 to 0.8% (vol). Oxygen and CO_2 measurements were normalized to a pressure of 800 mm Hg to account for any fluctuations in atmospheric or vessel headspace pressure. The O_2 and CO_2 sensors were each calibrated at two concentrations spanning at least 50% of the measurement range using certified calibration gases (Scott Specialty Gases, Plumsteadville, PA, USA). Calibration of the sensors was performed immediately prior to initiation of each test and was verified at the conclusion of each test to demonstrate consistency in measurements over the duration of the experiments.

Quantitative analyses of BPA in the low-level river dieaway study were performed using a Hewlett-Packard (HP) model 5989 gas chromatograph (Avondale, PA, USA) coupled to a mass selective detector (MSD). The analytical method employed in the study was based on the procedure developed and validated by Rinken (M.A. Rinken, unpublished data) and was shown to have the desired sensitivity, accuracy, and precision for the determination of BPA in the river water matrix used in this study. Samples were prepared for analysis as described above. A 1-μl aliquot of the extract was injected into the instrument using a Hewlett-Packard model 7673 auto sampler using a 30-s splitless injection with an injection port temperature of 280°C. Analytes were separated using a J&W DB-5MS (30 m × 0.25 mm, 0.5-μm film thickness; Alltech, Deerfield, IL, USA) capillary column with helium carrier gas at a column head pressure of 25 psi. The GC oven was temperature programmed from 80°C to 280°C at 10°C/min and the gas chromatography/mass selective detector (GC/MSD) transfer line temperature was maintained at 280°C. The MSD ion source was operated in the electron impact ionization (EI) mode at 70 eV at a temperature of 250°C. The MSD analyzer was operated in the selected ion monitoring (SIM) mode at a temperature of 120°C. The electron multiplier was operated at ~1,500 V. The MSD method was programmed to monitor ions at m/z of 420 (M^+ of BPA-TFA), 405 ($[M - CH_{3+}]^+$ of BPA-TFA), 428 (M^+ of D_8-BPA-TFA), and 413 ($[M - CH_3]^+$ of D_8-BPA-TFA). All ions were monitored at dwell times of 0.1 s/ion/scan. The concentration of BPA in the samples was calculated based on a linear regression equation constructed from the peak area ratio of the base peak ions ($[M - CH_3]^+$) for the analyte and internal standard (m/z 405 to 413) versus the known BPA standard concentration. The confirmation of BPA was performed by comparing the peak area ratio of M^+ and $[M - CH_3]^+$) ions (m/z 420 to 405) for standards and samples.

Data analysis

When determining the kinetics of biodegradation, the lag time was defined as the period during which changes in BPA concentration were less than 10% of the initial test chemical concentration. Kinetics of BPA degradation (after the lag phase) were determined by plotting the natural logarithm of the BPA concentration as a function of time. Pseudo–first-order biodegradation rate constants (k) were determined by regression analysis and corrected for any losses observed in the killed controls. The half-life for BPA biodegradation was calculated using the relationship $t_{1/2} = \ln(2/k)$.

With the respirometric experiments, the lag time prior to BPA biodegradation was defined as the time required for the biochemical oxygen demand (BOD) in the reaction mixtures to exceed 10% of the theoretical oxygen demand (ThOD) for the added BPA. Calculations of the ThOD and maximum theoretical CO_2 yield ($ThCO_2$) for BPA were based on the measured concentrations of BPA added to the test mixtures as determined by HPLC analysis. For simplicity, the half-life for BPA in the respirometric experiments was defined as the time (in days) required beyond the lag time for the BOD to exceed 50% of ThOD. Since increases in nitrite and nitrate concentrations in the test mixtures (corrected for blanks) during the experiments were typically less than 0.5 mg/L, oxygen consumption results were not corrected for nitrification of ammonia.

RESULTS

River water and sediment characterizations

Rivers for the study were selected on the basis of geographical locations (United States vs Europe), northern versus

southern climates, industrial versus rural locations, and freshwater versus estuarine environments. Characteristics of the water and sediment samples used in the ^{14}C-die-away and respirometric studies are summarized in Tables 1 and 2. The BPA was not detected in the river water samples prior to addition of the test compound (detection limits for the HPLC method used ranged from 50 to 100 μg/L). River water pH ranged from 6.9 to 8.3, while in situ water temperatures ranged from 7.7 to 30.6°C. Dissolved oxygen concentrations in the water samples ranged from 4.8 to 12.4 mg/L, indicating that the water column was aerobic at each of the collection sites. Conductivity measurements ranged from 86 μS/cm for the Ware River to 19,800 μS/cm for the estuarine waters of the Westerschelde River and were consistent with total dissolved solids measurements in the range of nondetected to 18,500 mg/L. Heterotrophic bacteria in the water samples (as measured by plate counts on standards methods agar) ranged from 2.6×10^1 to 3.7×10^4 colony forming units (CFU)/ml.

Composite sediment samples were prepared from upstream and downstream material collected from the different rivers. Of the nine composite samples, five were classified as sands, three samples were sandy loams, and one sample was characterized as a loam (Table 2). Organic carbon content of the sediments varied from 0.06 to 2.0%. Heterotrophic bacterial populations as measured by plate counts ranged from 9.3×10^4 to 2.1×10^6 CFU/g.

Fate of BPA in river water

Die-away studies for [^{14}C]BPA in shake flasks were conducted in parallel with respirometer studies for Rhine and Ohio river waters to determine the applicability of the respirometer for measuring BPA biodegradation rates in surface waters. Similar results were obtained with both experimental approaches, as illustrated in Figure 1. Following a lag period of 4 to 6 d, rapid biodegradation of BPA was observed in both test systems. In the ^{14}C-die-away studies, 50% degradation occurred in less than 2 d, and concentrations of [^{14}C]BPA reached nondetected levels (<7 μg/L) within 3 d following the onset of biodegradation. In the respirometer studies, 50% degradation (based on oxygen consumption) of 5,000 μg/L BPA occurred within 1 d in the same water sample following a 4-d lag phase. Mineralization of BPA to CO_2 was similar in both test systems. Mineralization of 5,500 μg/L [^{14}C]BPA to $^{14}CO_2$ reached 80% in the shake flask microcosms after 25 d (data not shown), as compared with 81% conversion to CO_2 after 17 d from the biodegradation of 5,000 μg/L BPA in the respirometer (Fig. 1).

As summarized in Table 3, BPA biodegradation in shake flask and respirometer experiments was similar for both the Rhine and Ohio Rivers and for samples taken both upstream and downstream of wastewater treatment plant outfalls. Following lag periods ranging from 2 to 8 d, BPA was rapidly degraded in both Rhine and Ohio river waters with half-lives in the range of 0.5 to 1.4 d. The BPA was extensively degraded, as levels of CO_2 recovered ranged from 65 to 91%.

Negligible losses of BPA were observed in autoclaved (killed) controls, confirming that the degradation observed in the viable microcosms was biologically mediated (Table 3). In killed controls, recovery of [^{14}C]BPA was >92% after 18 d in the Rhine River waters and >99% after 14 d in the Ohio River waters. The formation of [^{14}C]metabolites or $^{14}CO_2$ was not detected in the controls. With the respirometer studies,

Table 1. Characterization of river water samples

River	Site	Location	Temperature (°C)	Dissolved oxygen (mg/L)	pH	Conductivity (μS/cm)	Total dissolved solids (mg/L)	Plate count (colony forming units/ml)
Rhine, industrial (Germany)	Upstream	51°N; 6°E	23.9	10.1	6.9	610	0	5×10^1
	Downstream		24.8	10.6	7.1	580	10	2.6×10^1
Ohio, industrial (Indiana, USA)	Upstream	38°N; 88°W	27.3	7.4	8.2	471	293	2.4×10^3
	Downstream		27.4	7.8	8.3	474	297	7.2×10^3
Westerschelde, industrial–estuarine (The Netherlands)	Upstream	51°30′N; 4°E	12.8	8.0	7.6	10,200	8,320	1.9×10^2
	Downstream		11.8	9.1	7.9	19,800	18,525	2.3×10^2
Ware, rural–light industry (Massachusetts, USA)	Upstream	42°N; 72°W	7.7	12.1	7.0	86	56	3.6×10^2
	Downstream		8.2	12.4	7.0	87	57	5.8×10^2
Monte Sano Bayou, industrial (Louisiana, USA)	Upstream	30°30′N; 91°W	23.8	4.8	7.4	491	319	5.8×10^2
	Downstream		30.6	5.7	7.3	3,200	2,080	3.7×10^4
Mississippi, industrial (Louisiana, USA)	Not applicable	30°30′N; 91°W	14	—[a]	7.7	477	310	2.7×10^3
Elbe, industrial–estuarine (Germany)	Upstream	53°30′N; 9°W	—[a]	—[a]	8.0	1,544	1,004	7.9×10^3
	Downstream		7.8	7.9	7.8	2,030	1,319	5.3×10^3

[a] Not measured.

Table 2. Characterization of sediment samples (see Table 1 for river locations)

River	Site	% Sand	% Silt	% Clay	Classification	pH	Cation exchange capacity	Organic carbon (%)	Plate count (colony forming units/g)
Rhine	Upstream	74	14	12	Sandy loam	8.1	10.1	0.06	1.5×10^6
	Downstream	98	2	0	Sand	8.3	5.0	0.52	3.9×10^5
Ohio	Upstream	97	1	2	Sand	7.9	2.0	0.23	1.6×10^5
	Downstream	96	2	2	Sand	8.0	1.3	0.23	1.2×10^6
Westerschelde	Upstream	72	20	8	Sandy loam	7.9	14.4	1.0	3.8×10^5
	Downstream	70	20	10	Sandy loam	8.1	30.6	2.0	9.3×10^4
Ware	Upstream	91	6	3	Sand	6.2	1.9	1.0	5.9×10^5
	Downstream	94	4	2	Sand	6.8	1.3	0.28	3.6×10^5
Monte Sano Bayou	Upstream	40	38	22	Loam	7.9	15.7	1.2	2.1×10^6

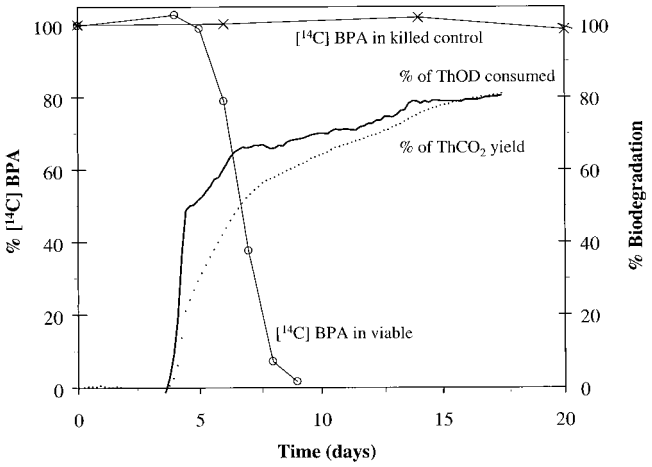

Fig. 1. Biodegradation of bisphenol A (BPA; 5,500 μg/L) in Rhine River water (downstream sample) in both ^{14}C-die-away (○—○; ×—×) and respirometer studies (– – –; ·····). Results represent the average of duplicate test mixtures. Results of the respirometer studies are expressed in terms of percent of the theoretical oxygen demand (% ThOD) consumed and the percent of the theoretical carbon dioxide (% $ThCO_2$) yield and are corrected for the unamended controls.

negligible O_2 consumption and CO_2 production were observed in the killed controls.

The disparity between lag periods observed between the two experimental approaches was investigated further. For example, lag phases prior to the onset of BPA biodegradation in Rhine River waters ranged from 5 to 8 d in the shake flasks, as compared with 3 to 4 d in the respirometer (Table 3). Since the headspace gases of the shake flask microcosms had been enriched with pure O_2 gas while the respirometer microcosms contained ambient air, the role of oxygen on the lag phase was investigated. During subsequent studies with Ohio River water samples, shake flask microcosms were incubated with and without oxygen sparging of the headspace. For both upstream and downstream samples, the lag phase in microcosms containing air in the headspace (i.e., no O_2 sparging) was reduced from approximately 4 to 5 d to a range of 2 to 3 d (data not shown). The shorter lag phase was consistent with those observed in the respirometer microcosms for the same river water/sediment mixtures. Thus, enriching the headspace gases of the microcosms with pure O_2 gas appeared to increase the lag phase for BPA biodegradation.

The HPLC-RAM analysis of the aqueous phase of the reaction mixtures with time showed disappearance of [^{14}C]BPA and formation of [^{14}C]metabolites, which eluted at the solvent front (Fig. 2). Chromatographic analysis of this radioactivity indicated that the material was polar and water soluble and was not resolved from standards of [^{14}C]carbonate analyzed in an analogous manner. When selected river water samples were acidified and reanalyzed by HPLC-RAM, between 82 and 92% of this residual radioactivity was lost from peaks eluting at the solvent front (presumably due to volatilization). These results confirm that [^{14}C]CO_2 was the predominant degradation product, which tended to remain dissolved in the reaction mixtures. The HPLC-RAM analysis of the residual radioactivity remaining in the river water after acidification showed no defined chromatographic peaks (Fig. 2C). Since the HPLC-RAM system would have been able to detect a metabolite that represented 1.5% of the parent compound, these observations suggest that [^{14}C]BPA was converted to a complex mixture of polar metabolites (which do not chromatograph as

Table 3. Summary of bisphenol A biodegradation in river water (see Table 1 for river locations)

River	Site	Colony forming units/ml	14C-die-away studies				Respirometer studies				
			[BPA] (μg/L)	Lag[a] (days)	$t_{½}$ (days)	Max % $^{14}CO_2$	[BPA] (μg/L)	Lag (days)	$t_{½}$ (days)	Max O_2 (% ThOD)	Max CO_2 (% $ThCO_2$)
Rhine	Upstream	50	46	6–7	0.9	77 ± 2					
	Upstream	50	5,560	8	1.2	77 ± 4	5,070	3.5	0.7	100 ± 2	91 ± 2
	Downstream	50	46	5–6	0.8	73 ± 3					
	Downstream	50	492	5–6	0.5	77 ± 2					
	Downstream	50	5,650	5–6	0.6	80 ± 1	5,160	4.1	0.5	81	81
	Killed control	—	492	NA[b]	No degradation	None	500	NA	No degradation	None	None
Ohio	Upstream	2,400					5,370	2.3	0.5	84 ± 6	74 ± 6
	Downstream	7,200	46	2–5	1.4	65 ± 3					
	Downstream	7,200	5,980	5	0.9	70 ± 3	5,460	2.9	0.5	80 ± 3	75 ± 4
	Killed control	—	46 and 5,520	NA	No degradation	None	5,000	NA	No degradation	None	None
Westerschelde	Upstream	190					4,470	3.3	2.0	88 ± 1	67 ± 3
	Downstream	230					4,880	4.2	1.2	72 ± 14	61 ± 10
	Killed control	—					5,000	NA	No degradation	None	None
Elbe	Upstream	7,930					4,130	2.6	0.5	94 ± 3	96 ± 4
	Downstream	5,270					2,780	2.9	1.0	90	103
	Killed control	—					5,000	NA	No degradation	None	None
Ware	Upstream	360					5,150	3.3	1.0	80 ± 6	69 ± 5
	Downstream	580					4,980	4.4	1.8	80 ± 8	72 ± 6
	Killed control	—					5,500	NA	No degradation	None	None
Monte Sano Bayou	Upstream	580					5,060	3.4	1.0	75 ± 4	59 ± 1
	Downstream	37,000					5,160	4.0	2.6	53 ± 27	—
	Killed control	—					5,500	NA	No degradation	None	None
Mississippi	NA	2,700					4,750	2.8	1.7	84 ± 6	74 ± 7
	Killed control						5,500	NA	No degradation	None	None
				Mean ± SD =	0.9 ± 0.3		Mean ± SD =	3.4 ± 0.7	1.2 ± 0.7		

[a] Longer lag periods in ^{14}C-die-away studies shown to be an artifact of experimental design (O_2 sparging).
[b] NA = not applicable; SD = standard deviation.

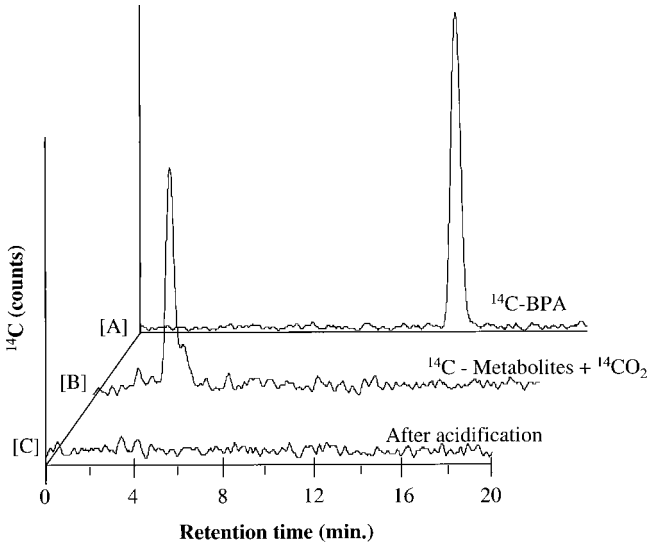

Fig. 2. Representative high performance liquid chromatography radiochromatograms of Rhine River water samples incubated with 5,500 μg/L [^{14}C]-bisphenol A ([^{14}C]BPA) at day 0 (A), day 11 (B), and day 11 after acidification (C).

unique entities), as would be expected for the entry of carbon-14 from the test compound into the metabolic cycles of the microorganisms, as well as incorporation into new microbial biomass.

Kinetics of BPA biodegradation

As illustrated in Figure 3, the degradation of [^{14}C]BPA in the shake flask microcosms was consistent with first-order kinetics since the slope of the biodegradation curves and corresponding pseudo–first-order rate constants were relatively uniform over the range of initial test chemical concentrations (50–5,500 μg/L). Half-lives for BPA ranged from 0.5 to 1.2 d in Rhine River waters, compared with 0.9 to 1.4 d in the Ohio River waters and were independent of BPA concentration (Table 3). Half-lives for BPA determined in the respirometer studies (i.e., time after the lag period required for oxygen consumption to reach 50% of ThOD) were similar and ranged from 0.5 to 0.7 d for both river waters (Table 3).

The fact that BPA degradation kinetics appeared to be first

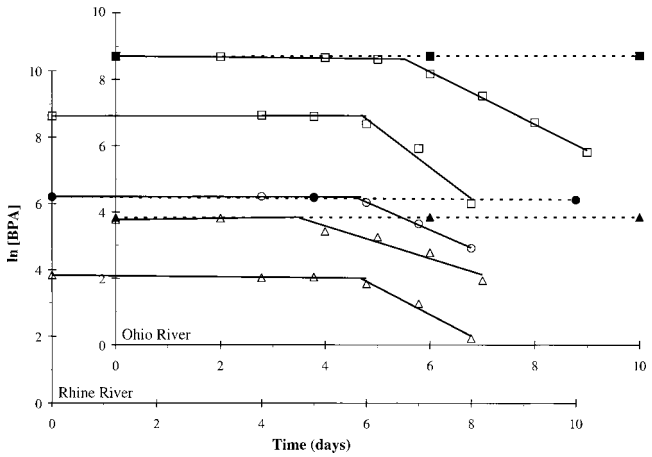

Fig. 3. Kinetics of [^{14}C]-bisphenol A ([^{14}C]BPA) biodegradation in Rhine and Ohio river waters. Changes in [^{14}C]BPA concentration in active microcosms (open symbols) and killed controls (closed symbols).

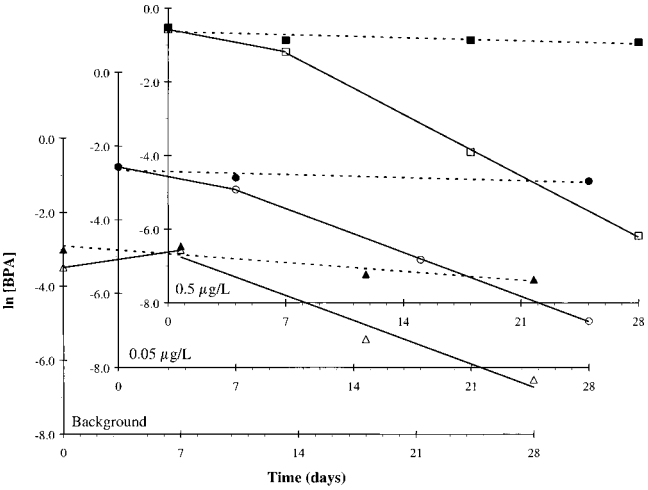

Fig. 4. Kinetics of bisphenol A (BPA) degradation at trace levels in Mississippi River water. Changes in BPA concentration in active microcosms (open symbols) and killed controls (closed symbols).

order over the range of concentrations examined in the study, combined with the good agreement between the two experimental approaches, suggested that the respirometer was suitable for determining half-lives for BPA degradation. Consequently, the biodegradation of BPA was examined in five additional river waters using the respirometer system alone. Results are summarized in Table 3. Although the river waters were collected from diverse locations, the results were remarkably similar. Lag times prior to the onset of BPA biodegradation averaged 3.4 ± 0.7 d, while half-lives averaged 1.2 ± 0.7 d. The kinetics of degradation in the various water samples did not appear to correlate with the size of the microbial population since the total numbers of heterotrophic bacteria as measured by plate counts varied by nearly three orders of magnitude. Furthermore, little difference was observed in lag times or half-lives for BPA degradation in samples collected either upstream or downstream of wastewater treatment systems known to receive BPA.

Kinetics of BPA biodegradation at trace levels

A low-level die-away study was performed to determine if half-lives measured at initial BPA concentrations ranging from 50 to 5,500 μg/L were applicable for describing the biodegradation kinetics at nanogram-per-liter concentrations (50–500 ng/L). Because of analytical limitations, only primary biodegradation (loss of parent) was monitored in river water alone. Results of the study are illustrated in Figure 4. Analysis of viable microcosms on day 0 using the GC/EI/MS method indicated that background BPA concentrations present in the river water were in the range 0.03 ± 0.001 μg/L.

After 28 d of incubation, BPA concentrations in all of the viable microcosms were reduced to below the 0.005 μg/L method limit of quantitation. The method limit of quantitation was validated during recovery experiments using control river water samples fortified at the 0.005 μg/L level. However, the low levels of BPA that could be detected in the 28-d samples were useful for the determination of biodegradation rate constants. These data were included based on a method limit of detection of 0.000232 μg/L, which was calculated at 10 times the instrument noise observed at the BPA (ion 405) retention time.

Pseudo–first-order rate constants for the disappearance of

Table 4. Biodegradation of bisphenol A (5,000 μg/L) in river water + 0.05% sediment mixtures (respirometer studies); CFU = colony-forming units; SD = standard deviation; see Table 1 for river locations

River	Site	CFU/ml in reaction	Lag (days)	$t_{½}$ (days)
Rhine	Upstream	1,100	3.4	0.8
	Downstream	280	3.3	0.7
	Killed control	—	—	No degradation
Ohio	Upstream	2,500	2.6	0.8
	Downstream	7,900	2.6	0.8
	Killed control	—	—	No degradation
Westerschelde	Upstream	490	3.4	1.5
	Downstream	310	4.6	1.0
	Killed control	—	—	No degradation
Ware	Upstream	790	4.1	3.4
	Downstream	810	3.2	1.2
	Killed control	—	—	No degradation
Monte Sano Bayou	Upstream	2,200	3.2	0.8
	Killed control	—	—	No degradation
		Mean ± SD =	3.4 ± 0.6	1.2 ± 0.9

BPA were determined from plots of the natural logarithm of the BPA concentration versus time during the degradation phase for each of the BPA concentrations evaluated (Fig. 4). For the purposes of this calculation, the degradation phase was defined as occurring between days 7 and 28. Day 0 values were not included in this calculation to avoid including the lag phase, which occurs between days 0 and 7 (prior to the onset of biodegradation). Rate constants for abiotic losses were determined from analysis of the results of the killed controls. Pseudo–first-order biodegradation rate constants were determined by subtracting the abiotic rate constant from the overall rate constant for the disappearance of BPA in the viable microcosms. Based on these rate constants, the biodegradation half-lives were 2.9, 4.2, and 5.6 d for measured BPA concentrations of 0.57, 0.077, and 0.03 μg/L (background), respectively.

Effects of sediment on BPA biodegradation

The effects of sediment concentration on BPA degradation were investigated with the respirometer in reaction mixtures prepared with combinations of river water and surface sediments. The addition of 0.05% sediment (dry wt) to the river water samples had little effect on the rapid degradation of BPA. As summarized in Table 4, the average lag periods and half-lives for the biodegradation of BPA in water/sediment mixtures (Table 4) were 3.4 ± 0.6 d and 1.2 ± 0.9 d, respectively, and are almost identical to the results obtained for river water alone (3.4 ± 0.7 d and 1.2 ± 0.7 d; Table 3). The fact that 0.05% sediment loadings had negligible effect on the rapid degradation of BPA can be partly explained by comparing the heterotrophic bacterial counts calculated for reaction mixtures prepared with water and sediment (Tables 3 and 4). For most of the reaction mixtures, the addition of 0.05% sediment increased the total bacterial count by less than a factor of four.

The effects of elevated sediment concentrations on the biodegradation of [^{14}C]BPA are shown in Figure 5. The addition of 10% sediment to Rhine River microcosms decreased both the lag period and the time required for complete degradation compared with microcosms prepared with river water alone. These results are consistent with the increased size of the microbial population, from 50 CFU/ml in water alone to 4.7 × 10^4 CFU/ml in microcosms containing 10% sediment. In the 10% sediment microcosms, the lag phase decreased from about 5 d to less than 2 d, while concentrations of [^{14}C]BPA in the reaction mixtures were completely reduced to nondetectable levels in 6 d. These losses were due to biological activity and not adsorption since over 90% of the parent compound could be recovered from killed controls containing 10% sediment after 18 d. However, while degradation of the parent compound was increased, the mineralization of [^{14}C]BPA in microcosms containing 10% sediment was reduced, as levels of $^{14}CO_2$ production reached only 16% after 21 d of incubation; negligible mineralization (<2% $^{14}CO_2$) was observed in the killed controls. In the biologically active samples, approximately 8% of the radioactivity remained in solution following acidification and $^{14}CO_2$ analysis, while the majority of the remaining radioactivity was strongly bound (perhaps covalently) to the sediment since it could not be extracted with organic solvents. Combustion was required to recover the radioactivity bound to the sediment (~47 ± 6% of the total radioactivity added).

DISCUSSION

River die-away studies with BPA using a wide variety of surface waters have confirmed the rapid biodegradability of the compound, with an average half-life of approximately 1 d. The sampling sites included both northern and southern locations in the United States and Europe, both freshwater and

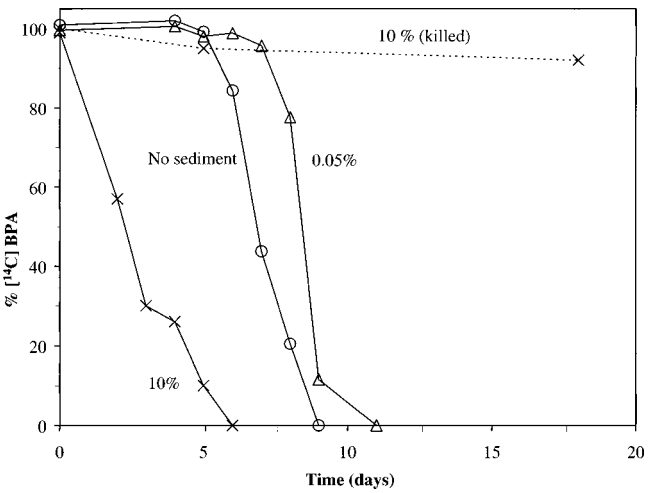

Fig. 5. Effect of sediment concentration on biodegradation of 500 μg/L [^{14}C)-bisphenol A ([^{14}C]BPA) in Rhine River water.

estuarine environments, as well as those from light and heavily industrialized rivers. Similar results for the degradation of BPA in surface waters have been reported by others [10,19]. For example, Dorn et al. [10] found greater than 95% primary biodegradation of 3,000 μg/L BPA within 3 to 5 d in effluent and receiving waters from a BPA manufacturing facility. Jin et al. [19] reported BPA was rapidly degraded in surface water samples taken from multiple sites on seven Japanese rivers. The rapid degradation of BPA in a broad range of surface waters suggests that microorganisms capable of degrading BPA are ubiquitous in the aquatic environment.

The biodegradation of [^{14}C]BPA in the shake flask microcosms appeared to follow first-order kinetics since the pseudo–first-order rate constants and corresponding half-lives were relatively uniform over the range of initial test chemical concentrations (50–5,500 μg/L). However, an important consideration of the study design was the ability to extrapolate biodegradation kinetics over a range of environmentally relevant BPA concentrations. Results of recent monitoring studies conducted in Germany and Japan have suggested that average environmental concentrations are on the order of 0.05 μg/L [16,17]. These findings prompted additional die-away studies at test chemical concentrations of 0.05 to 0.5 μg/L, which showed that half-lives at these trace levels (3–6 d) were comparable with those observed at the higher concentrations (50–5,500 μg/L). Consequently, biodegradation half-lives determined in this study can be used to provide reasonable estimates of the biodegradation kinetics at the trace concentrations found in surface waters.

The lag periods prior to the biodegradation of BPA were relatively short (2–4 d) for the range of surface waters examined in the study and are consistent with those described by others [10,19]. Furthermore, because similar degradation rates were observed in water samples collected both upstream and downstream of sewage treatment facilities that receive BPA-containing wastewaters, rapid degradation of BPA does not appear to be dependent on prior exposure to the compound. These observations suggest that microbial communities in environmental samples have the intrinsic ability to adapt quickly for BPA degradation. While lag periods for the degradation of [^{14}C]BPA in shake flask experiments appeared to be slightly longer than those observed in the respirometer for the same water sample, the disparity was shown to be an artifact of the experimental design. The longer lag periods in the shake flasks were traced to exchanging the air in the headspace of the microcosms with pure oxygen. The reason for this increased lag period remains unclear and its explanation is beyond the scope of the study.

Half-lives for BPA degradation were relatively constant over the broad range of surface waters examined in the study. In contrast, the total number of heterotrophic bacteria in the water samples as measured by plate counts varied by nearly three orders of magnitude. While the addition of small amounts of sediment (0.05%) had negligible effects on BPA degradation rates, the addition of 10% sediment to the microcosms substantially decreased both the lag period and the time for complete degradation of the parent compound. The effects of high sediment concentrations on biodegradation were likely due to an increase in the number of BPA-degrading microorganisms present in the microcosms. Although the correlation between BPA biodegradation kinetics and the number of heterotrophic bacteria is unclear, these observations suggest that a considerable fraction of the microorganisms present in aquatic environments have the ability to degrade the compound.

Degradation of [^{14}C]BPA in river water resulted in complete mineralization to [^{14}C]CO_2. The accumulation of by-products was not detected. Although HPLC analysis of river water samples with time showed disappearance of [^{14}C]BPA and formation of [^{14}C]metabolites that eluted at the solvent front, further analysis confirmed that the product was [^{14}C]carbonate. In contrast, Jin et al. [19] reported that, while BPA was rapidly degraded in water samples from seven Japanese rivers, the accumulation of metabolites was apparent in a number of the microcosms since more than 10% of the initial total organic carbon (TOC) remained in the samples at the completion of the studies. Because it is difficult to differentiate between metabolites of BPA and by-products of intermediary cellular metabolism using TOC analysis, the validity of these conclusions is uncertain. Further, the accumulation of recalcitrant metabolites due to incomplete degradation of BPA is not supported by the results of the present study with [^{14}C]-labeled test material.

The complete degradation of BPA is consistent with the ability of microorganisms to utilize the compound as a sole carbon source for growth. Lobos et al. [6] and Spivak et al. [7] have previously described the pathway for BPA degradation by a gram-negative bacterium, designated strain MV1. They reported degradation of BPA proceeded via two pathways. The major pathway involved oxidation and cleavage of the propane functionality to form *p*-hydroxyacetophenone and *p*-hydroxybenzaldehyde, both of which were further degraded to CO_2 and water or were incorporated into biomass. A minor degradation route resulted in the formation of 2,3-bis(4-hydroxyphenyl)-1,2-propanediol and *p*-hydroxyphenacyl alcohol. Jin and coworkers [20] reported a similar metabolic pathway for BPA by *Pseudomonas paucimobilis* strain FJ-4. While the results of pure culture studies are useful for elucidating metabolic pathways, they may not reflect the ultimate fate of chemicals in the environment where degradation is due to the concerted activities of diverse microorganisms within the microbial community. Results of the present study with [^{14}C]BPA indicate that any metabolites that are formed are subject to further degradation since they did not accumulate in the test system.

The rapid and extensive mineralization of BPA to CO_2 in surface waters observed in the present study is consistent with results of standardized ready biodegradation tests [4,21]. West et al. [4] reported that BPA was rapidly degraded in an OECD ready biodegradation test, as indicated by 28-d oxygen consumption and CO_2 production of greater than 80 and 76%, respectively. Similarly, respirometric studies with five river waters showed an average 76% oxygen consumption and 67% CO_2 production within 15 to 20 d for biodegradation of BPA. These observations support the widely held assertion that chemicals that are shown to pass a ready biodegradation test are unlikely to persist in the environment [22].

The present study has demonstrated the rapid and complete biodegradation of BPA in surface waters and sediments from seven different locations in the United States and Europe. These observations, combined with previous results from standardized biodegradation tests as well as both wastewater and environmental simulation studies, provide clear evidence that BPA is not persistent in the aquatic environment.

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