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DEGRADATION OF NONYLPHENOL ETHOXYLATES IN ESTUARINE SEDIMENT UNDER AEROBIC AND ANAEROBIC CONDITIONS

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Abstract—Nonylphenol ethoxylate (NPEO) surfactants and their metabolites are ubiquitous contaminants of the aquatic environment. Despite considerable interest in the environmental fate of these compounds due to concerns over toxicity and estrogenic activity, the pathways of NPEO degradation in sediments have not previously been reported, in spite of the fact that sediment appears to be an important sink for these compounds in the environment. In the present work, we have examined the rates and pathways of NPEO degradation in batch sediment slurry experiments using radiolabeled NPEO mixtures. Results suggest that NPEOs are more persistent in sediments under anaerobic conditions than in the presence of oxygen. In addition, it was illustrated that NPEO degradation proceeds via separate pathways in oxic and anoxic sediment. Discernible metabolites were identified and an overall mass balance for NPEO degradation in oxic and anoxic sediment was achieved. In contrast with previous studies, no evidence was observed for net production of nonylphenol from NPEOs during aerobic or anaerobic degradation. The observed relative rates at which NPEO ethoxymers disappeared in the sediment slurry experiments were consistent with previous reports for these compounds in sediment and other environmental media, although the absolute rates measured were somewhat faster than those reported for field sediments.

Keywords—Alkylphenol Electrospray Carboxylate Fate Metabolite

INTRODUCTION

Nonylphenol ethoxylates (NPEO, Fig. 1) are high production volume (U.S. sales of 225 metric tonnes in 1994 [1]) synthetic nonionic surfactants used in detergent formulations, industrial cleaners, and emulsifiers. While NPEOs are very effective industrial surfactants, recent regulatory attention has been focused on these compounds due to concerns over their biodegradability, persistence in the environment, and the potential toxicity of some of their metabolites to aquatic organisms. In addition, exposure to some metabolites of NPEOs, most notably nonylphenol (NP), has been found to produce estrogenic effects in aquatic organisms [2].

Biological wastewater treatment has been reported to remove >90% of the NPEO burden of sewage influent [3]; however, the remaining NPEO mixture is typically dominated by relatively hydrophobic compounds with short ethoxy chains such as nonylphenol (NP, Fig. 1) and nonylphenol mono-, di-, and triethoxylates (NP(1–3)EO, Fig. 1) [4]. Discernible NPEO metabolites, such as nonylphenol carboxylates (NPECs, Fig. 1) [5] and dicarboxylated alkylphenol ethoxylates (CAPECs, Fig. 1) [6] may also be observed in biologically treated wastewaters. Even with removal efficiencies >90% during wastewater treatment processes, NPEOs have been shown to enter and persist in the aquatic environment [7,8]. Within aquatic systems, NPEOs and NP (which for the present purposes will be considered as part of the NPEO series, i.e., NP0EO) have been observed to concentrate in sediments [9–11] due to their relatively high particle reactivity [12].

Although there have been several studies of NPEO biodegradation behavior in natural waters [13–16], only a single report exists for the degradation of this compound class in natural sediments under laboratory conditions [17]. However, that study focused only on the NPEO metabolite, NP. To the best of our knowledge, no data are available detailing the biodegradation behavior of polyethoxylated NPEOs in aquatic sediments.

In the present work, we report on the biodegradation behavior of NPEOs in oxic and anoxic slurries of highly sewage-impacted estuarine sediment. The objectives of the study were to trace the disappearance of individual NPEOs and the appearance of NPEO metabolites and degradation products under aerobic and anaerobic conditions, to examine relative biodegradation rates among individual NPEO ethoxymers in oxic and anoxic sediment slurries, and to decipher the pathways of NPEO biodegradation in sediments under aerobic and anaerobic environmental conditions. To this end, incubations of sediment slurries spiked with ^{14}C-labeled NPEOs were performed under aerobic and anaerobic conditions in the laboratory over a period of 116 (aerobic) or 129 (anaerobic) d, and the disappearance of NPEOs and emergence of radiolabeled metabolites were traced over the time course of the experiment.

EXPERIMENTAL SECTION

Materials

The [$^{14}C_6$]-NPE4 mixture used for biodegradation experiments was a kind gift of the Alkylphenol Ethoxylate Research Council (Washington, DC, USA). This material had been specially synthesized to reproduce the branched alkyl isomer mixture characteristic of commercial NPEO formulations and contained a mixture of NPEO ethoxymers with an average ethoxy chain length of 4. The specific activity of the [$^{14}C_6$]-NPE4 was 29.2 mCi/mmol, and the ^{14}C label was located on the aromatic ring (all six carbons) of the nonylphenol moiety. All solvents used in sample preparation and analysis were high-performance liquid chromatography (HPLC) grade or equivalent.

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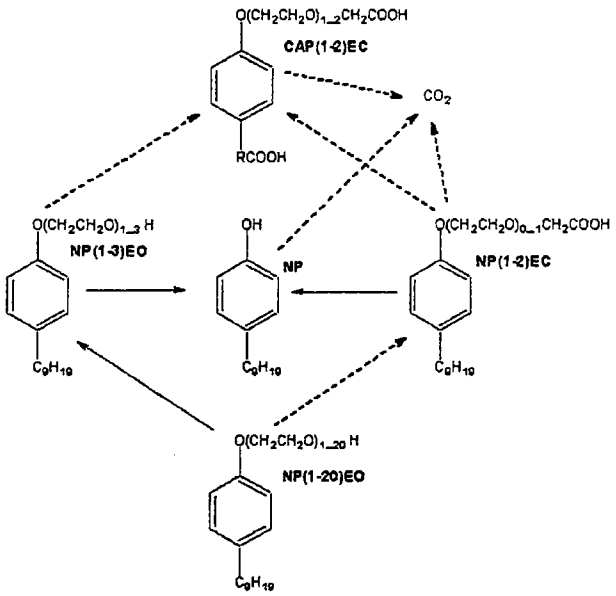

Fig. 1. Structures of nonylphenol ethoxylates (NPEO) and their metabolites, including nonylphenol (NP), nonylphenol carboxylates (NPEC), and alkylphenol dicarboxylates (CAPEC). Numbers in parentheses denote the range in ethoxy chain lengths. Microbial biotransformation pathways reported in the literature (see text for references) and in the present study are shown, including ethoxy chain shortening (solid lines) and oxidation (dashed lines).

Scintillation cocktails were obtained from Packard Instrument (Hionic-Fluor, Insta-Fluor Plus; Downers Grove, IL, USA) and R.J. Harvey Instrument ($^{14}CO_2$ trapping cocktail; Hillsdale, NJ, USA).

Biodegradation experiments

Surface sediment (top ~5 cm) was collected on May 17, 2001, in 15 m of water from a depositional site within Jamaica Bay (NY, USA) using a Smith-McIntyre grab sampler. The particular site within Jamaica Bay chosen for sediment collection (Grassy Bay) has been extensively studied with regard to hydrography [18], geochemistry [19], and NPEO fate [11,20] and is situated proximal to the outfall of a major wastewater treatment plant. The collected sediment was anoxic at all depths, with porewater pH = 7.4, oxidation-reduction potential (E_h) = −156 mV, and salinity = 26 practical salinity units (psu). After returning to the laboratory, the sediment was stored in sealed glass jars at 4°C prior to use in biodegradation experiments in July of 2001.

Prior to the start of the experiments, an aliquot (~500 g) of sediment was suspended in 1.8 L of filtered (<0.45 μm) artificial seawater (FSW; salinity = 26 psu) and oxidized by sparging with air for 5 d. This step was used to reduce the chemical oxygen demand of the sediment and to minimize changes in the slurry pH due to sulfide oxidation. The oxic NPEO biodegradation treatments were prepared by adding 1 g wet wt (0.18 g dry wt) of this oxidized sediment to each of 24 autoclaved 125-ml glass serum bottles, followed by 10 ml of oxygenated FSW (salinity = 26 psu). Each bottle was spiked with [$^{14}C_6$]-NPE4 (0.2 μCi in 10 μl of methanol), corresponding to an addition of approximately 2.6 μg NPEO for a spiked sediment concentration of 14 μg/g dry weight. Surface sediments from the Grassy Bay site have previously been found to be contaminated with NPEOs at concentrations >40 μg/g dry weight, with nearly all of this burden composed of NP and NP1EO [3]. After spiking, the bottles were capped with a Teflon®-lined butyl rubber stopper modified with a hanging base trap containing a folded cellulose filter paper (42.5 mm, Whatman qualitative No. 1; Clifton, NJ, USA). The bottle stoppers were then sealed with aluminum crimp caps. Six of the slurry bottles were spiked with 1% formaldehyde to eliminate biological activity prior to sealing. These treatments served as abiotic controls. All bottles were placed on an orbital shaker table in a dark incubator at 25°C.

Anoxic NPEO sediment slurry bottles were prepared in a similar manner to that described above for the oxic treatments. However, care was taken during the preparation to preserve an anaerobic environment prior to sealing the bottles. Sediment (not oxidized) was added to 13 autoclaved 125-ml serum bottles in an anaerobic glove box under N_2 atmosphere. To each bottle was added 10 ml of FSW (salinity = 26 psu) that had been stripped of O_2 by sparging with a N_2/CO_2 gas mixture adjusted to give a solution pH of 7.3. Bottles were capped and sealed prior to removal from the anaerobic glove box. Anoxic abiotic control treatments (three bottles) were spiked with 1% formaldehyde through the stopper septa. To start the degradation experiments, [$^{14}C_6$]-NPE4 (0.2 μCi in 10 μl of methanol, corresponding to approximately 2.6 μg NPEO) was added to each anoxic slurry bottle via the stopper septa, and the bottles were placed in an argon-flushed, airtight plastic container affixed to an orbital shaker table within a dark incubator at 25°C.

Slurry bottle sampling

Slurry bottles from the oxic and anoxic NPEO degradation experiments were sacrificed periodically for analysis. Bottles were removed from the incubator and either immediately processed, or frozen at −15°C pending analysis. Oxidation-reduction potential was measured in the slurries through the bottle septa using an 18-gauge needle platinum combination electrode with a Ag/AgCl reference (Microelectrodes, Bedford, NH, USA). Dissolved oxygen was measured through a stopcock airlock inserted into the bottle septa using an amperometric sensor (Microelectrodes) calibrated in aerated 26-psu FSW (0.21 atm O_2) and argon-sparged 26-psu FSW (0 atm O_2). $^{14}CO_2$ evolved in the slurry bottles over the course of the degradation experiment was trapped by adding 0.250 ml 4-N potassium hydroxide solution through the septa to the filter wick suspended by the base trap in the bottle headspace and then acidifying the slurry to pH <2 by addition of 0.200 ml 2-N HCl solution. The bottles were then placed back onto the orbital shaker at 25°C for 1 h. Bottles were then uncapped and base traps were removed and placed into 20-ml glass scintillation vials. Hionic-Fluor scintillant (15 ml) was added and the ^{14}C activity was assayed by liquid scintillation spectrometry (Tri-Carb 2100TR, Packard Instrument).

Acidified sediment slurries were transferred from the serum bottles to capped 16 × 150-mm glass round-bottom centrifuge tubes fitted with Teflon-lined screw caps. Slurries were centrifuged for 10 min at 3,500 rpm, and the overlying water was transferred to 15-ml conical glass centrifuge tubes with Teflon-lined caps. [$^{14}C_6$]-NPE4 and extractable metabolites were isolated from the remaining sediment ultrasonically (270 W ultrasonic bath, model FS-14; Fisher Scientific, Pittsburgh, PA, USA) with 4 × 3 ml methanol (25 min per extraction). Methanol extracts were evaporated to approximately 0.5 ml at 35°C under a gentle stream of N_2 and added to the previously collected water in the 15-ml conical glass centrifuge tubes. The

combined water/sediment extracts were then extracted sequentially with 3 × 2 ml of dichloromethane by wrist shaking. After centrifugation, the dichloromethane layers were removed from the test tubes, and combined sequential extracts were evaporated to dryness under a gentle stream of N_2 prior to analysis. The combined extraction method was found to recover 83% of added [$^{14}C_6$]-NPE4 in spiked sediment tests. ^{14}C activity remaining in the aqueous layer after the sequential dichloromethane extraction was determined by transferring 1 ml of this solution to a 20-ml glass scintillation vial, raising the pH to >7 with 4-N potassium hydroxide, adding 15 ml of Hionic Fluor cocktail, and counting by liquid scintillation spectrometry. Extracted sediment was dried and stored at 4°C prior to assay for bound, unextractable ^{14}C activity.

[$^{14}C_6$]-NPE4, metabolite analysis

Radiolabeled NPEO ethoxymers and extracted metabolites were resolved using normal phase HPLC and detected by flow-scintillation spectrometry. The HPLC system consisted of dual Shimadzu (Columbia, MD, USA) LC-9A high-pressure pumps, a Rheodyne 7125 manual injector with a 0.250-ml loop, a 250 × 3.2-mm PAC (Partisil, 10 μm) column from Phenomenex (Torrance, CA, USA), and a Shimadzu SPD-6AV ultraviolet absorbance detector. Mobile phases were 0.1% glacial acetic acid in hexane (A) and 4% glacial acetic acid in ethanol (B). A linear gradient of 5% B to 100% B in 15 min followed by a 10-min hold at 100% B was used for the analytical separation at a flow rate of 1 ml/min. The flow scintillation detector was a Radiomatic model A-200, with a 0.5-ml liquid flow cell. For ^{14}C detection, HPLC column effluent was mixed with a continuous 1.5 ml/min flow of Insta-Fluor Plus scintillant prior to entering the Radiomatic flow cell. ^{14}C counts were assayed every 4 s by the flow scintillation detector and transformed to disintegrations per minute using a quench correction curve constructed from an HPLC analysis of unlabeled sediment extract and a ^{14}C-toluene scintillant spike of known activity.

Sediment extracts (described above) were reconstituted in 0.200 ml of starting HPLC mobile phase, and 0.100 ml of this solution was injected onto the HPLC column. Peaks corresponding to [$^{14}C_6$]-NPEO ethoxymers in the [$^{14}C_6$]-NPE4 spiking standard and in the sediment extracts were identified by retention-time match with previously characterized, unlabeled mixtures of NP, NP1–3EO, and NP(0–15)EO (detected by ultraviolet absorbance at 280 nm after HPLC separation). Unknown metabolite peaks present in the chromatograms from sediment slurry extracts were identified by both retention-time match with authentic NP1EC and NP2EC standards and electrospray tandem mass spectrometry (ESI-MS/MS) analysis of collected fractions corresponding to the unknown peaks. The ESI-MS/MS analysis was conducted on a Micromass (Manchester, UK) Quattro I instrument using argon collision gas and a collision energy of 26 eV. The HPLC fractions, evaporated and reconstituted in 50:50 methanol:water, were infused into the ESI source at 10 μl/min using a syringe pump.

Determination of residual ^{14}C activity in extracted sediment

Sediment residue that had already been ultrasonically extracted for NPEOs as described above was combusted, and evolved $^{14}CO_2$ was trapped for scintillation analysis. This step was used to recover any [$^{14}C_6$]-NPE4-derived radioactivity that remained associated with the sediment matrix after solvent extraction. Dried sediment (~0.2 g) was placed on a porcelain crucible and combusted for 3 min at 900°C in a gas furnace oxidizer (Harvey Instrument Model OX600) with an O_2 flow of 350 ml/min. The $^{14}CO_2$ was collected by bubbling the gas flow through 15 ml of trapping scintillant cocktail and analyzed by liquid scintillation spectrometry.

RESULTS AND DISCUSSION

Geochemical conditions inside sediment slurry bottles

Periodic analyses for dissolved oxygen and E_h in oxic and anoxic sediment slurry bottles were performed. Oxygen remained undetectable in anoxic bottles throughout the experiment, and the E_h of the slurries was observed to settle at approximately −200 mV. At the start of the experiment, the pH of the anoxic slurry was 7.1. One month after the last slurry bottle sample was taken from the radiolabeled anoxic treatments (i.e., 167 d after the start of the experiment), the pH had risen to 8.0. These conditions, measured in the test slurry bottles, were not greatly different from those determined for the bulk sediment soon after field sampling (discussed above).

Dissolved oxygen declined gradually in oxic sediment slurry bottles during the course of the degradation experiment, from saturation (p_{O_2} = 0.21 atm) to approximately p_{O_2} = 0.14 atm at the end of the experiment. This decline was consistent with utilization of oxygen during aerobic microbial metabolism of organic material (including NPEOs) in the sediments. The E_h of oxic slurry bottles was observed to remain relatively constant at approximately 400 mV during the first 60 d of the degradation experiment, consistent with an oxidizing, aerobic environment. This was followed by a gradual increase in the E_h to approximately 750 mV by the end of the experiment. This E_h inflection in the oxic slurry bottles was likely related to a reduction in solution pH over time due to formation of organic and mineral acids in the bottles as a result of chemical and biological oxidation processes. The pH of the oxic slurries remained relatively constant at approximately 6.2 during the first 19 d of the experiment, but had dropped to 2.8 by one month after the end of the biodegradation experiment. It is likely that the greatest reduction in solution pH occurred after day 85, as it was during this time that the greatest increase in E_h was observed. Nevertheless, it is acknowledged that the observed changes in E_h and pH of the oxic slurries during the degradation experiment likely influenced the microbial population and geochemistry within these treatments. It is unlikely, however, that microbial activity was completely inhibited in the oxic sediment slurry bottles by low pH, even at the end of the experiment, as p_{O_2} continued to decrease steadily until the final sampling time.

Fate of NPEOs in sediment slurry incubations

Degradation of NPEOs was observed in both oxic and anoxic sediment slurries with no apparent lag period, although the extent and pattern of degradation differed greatly among the various NPEO ethoxymers and between oxic and anoxic treatments (Fig. 2). The mass balance calculated from recovered inventory of ^{14}C activity, derived from the initial [$^{14}C_6$]-NPE4 slurry bottle spike, averaged (± standard deviation) 89 ± 8% for oxic treatments and 96 ± 5% for anoxic treatments over the course of the biodegradation experiment. Nonylphenol was present at low levels (~5%) in the [$^{14}C_6$]-NPE4 spiking material and was observed to persist at these low levels

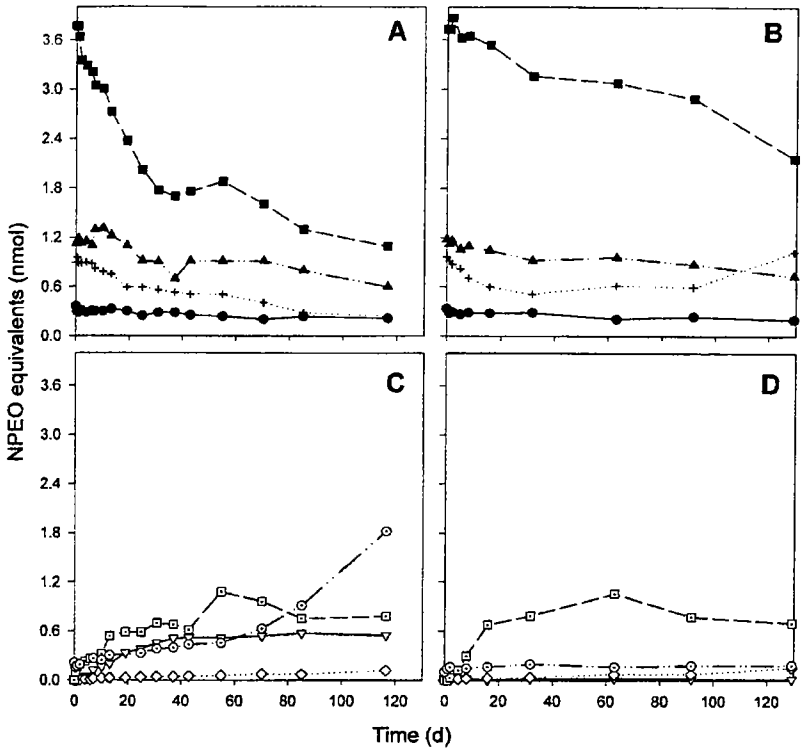

Fig. 2. Disappearance of nonylphenol (NP) (●) and nonylphenol ethoxylates (NPEO): NP1EO (+), NP2–5EO (■), and NP6–9EO (▲) with corresponding formation of nonylphenol ethoxycarboxylates (NP1–3EC, ⊡), $^{14}CO_2$ (◇), nonextractable dissolved ^{14}C (▽), and nonextractable sediment-bound ^{14}C (⊙) in oxic (**A**, **C**) and anoxic (**B**, **D**) sediment slurries over the course of the biodegradation experiment. Amounts (nmol) of NPEO and metabolites are given on a per-bottle basis. NPEO ethoxymers that were observed to exhibit similar trends over the time course of the experiment (i.e., NP2–5EO and NP6–9EO) are presented as groups in order to reduce the complexity of the figure.

throughout the degradation experiment in both oxic and anoxic treatments (Fig. 2A and B). At the end of the experiment, NP accounted for only approximately 3% of the initially added ^{14}C activity in both the oxic and anoxic treatments. Contrary to previous reports in soil, sludge, and wastewater [21–23], no clear evidence for net formation of NP as a metabolite of NPEOs was observed under anaerobic conditions in the present study. However, it is possible that the time scale of the experiment was insufficient for this process to become important, as in situ formation of NP from NPEO degradation has been interpreted to occur in anoxic sediment cores from the sediment collection site within Jamaica Bay, albeit over longer time scales [20]. It is also possible that small amounts of NP may have been formed and removed at similar rates during the present experiment, leading to the establishment of an apparent steady-state level of this compound in the sediment slurry bottles.

The NP1EO decreased in concentration throughout the oxic degradation experiment, falling from 13 to 3.5% of the initially added radioactivity (Fig. 2A). In contrast, a more complex behavior was observed for NP1EO in the anoxic slurry experiment (Fig. 2B). The NP1EO was observed to decline in concentration until after day 30 of the anoxic incubation, when a gradual increase in this ethoxymer was observed. At the end of the experiment, the NP1EO made up 15% of the initially added radioactivity in the anoxic treatment, nearly the same as at the beginning of the experiment (14%). The increase in NP1EO in anoxic slurry bottles after day 30 corresponded to a relative decrease in the concentration of NP2–5EO ethoxymers (Fig. 2B). It is likely that NP1EO was formed during the biodegradation experiment by scission of ethoxy groups from the NP2–5EO (Fig. 1), as has been previously reported for these compounds under anaerobic conditions [21,23]. At the end of the incubation period, NP2–5EO were together found to constitute 32% of the initially added ^{14}C activity in the anoxic slurry bottles, compared with 55% at the beginning of the experiment (Fig. 2B). The proportion of NP2–5EO present at the end of the experiment was much lower (16% of initially added ^{14}C activity) in the oxic treatments (Fig. 2A). The slight increase in NP2–5EO between 40 and 60 d in the oxic treatment was almost entirely due to an increase in a single ethoxymer, specifically NP3EO. Transient formation of this compound during aerobic NPEO biodegradation may have been analogous to the increase observed for NP1EO in the anoxic treatments. That is, NP3EO may have been formed as a degradation intermediate following the observed decrease in NP6–9EO (between days 15 and 40) during the biotransformation of these longer ethoxy-chain NPEOs (Fig. 1). This process has previously been reported to be important for NPEO biodegradation in some systems under aerobic conditions [4,11,14,24]. As was the case with NP, NPEOs with longer ethoxy chain lengths (NP6–9EO) were found to behave in a like manner under aerobic and anaerobic conditions, with some persistence evident (Fig. 2A and B). Collectively, these more highly ethoxylated compounds were found to account for 8.8 and 10.7% of initially added ^{14}C activity in oxic and anoxic slurry bottles, respectively, at the end of the biodegradation experiment, compared with 16 to 17%, respectively, at the beginning.

No formation or degradation was observed for any NPEO ethoxymers in either the oxic or anoxic killed control treatments. This suggests that all of the changes observed in NPEO

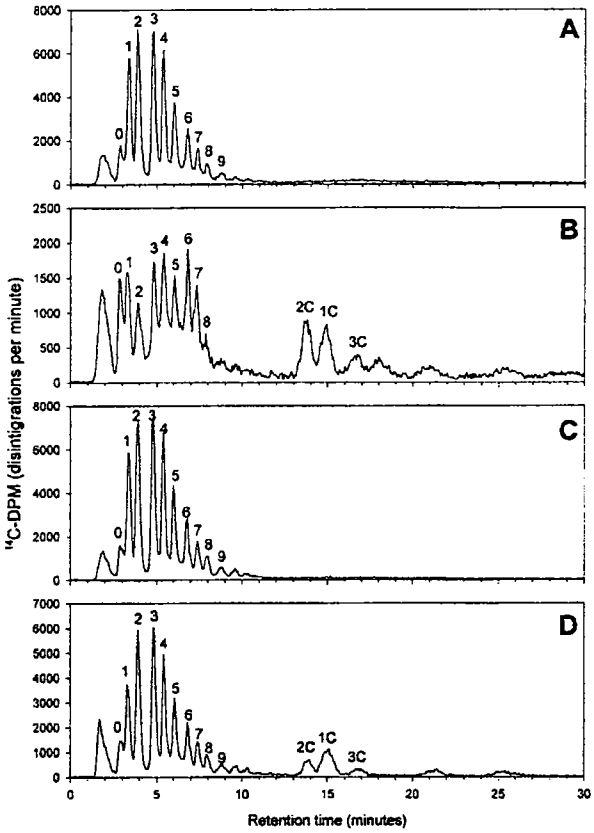

Fig. 3. Normal-phase high-performance liquid chromatography (HPLC) radiochromatograms of radiolabeled nonylphenol ethoxylates (^{14}C-NPEO) and metabolites in oxic killed control (**A**), oxic (**B**), anoxic killed control (**C**), and anoxic (**D**) slurry bottles after 85 (oxic) and 91 (anoxic) d of incubation. Numbered peaks correspond to NPEOs with the indicated ethoxy chain length. Nonylphenol ethoxycarboxylates (NPEC) are marked with their ethoxy chain length followed by the letter C.

concentrations with time in oxic and anoxic slurry bottles (Fig. 2A and B) were due to microbially mediated processes. The chromatographic distribution of NPEOs in the control treatments (Fig. 3) was identical to that of the [$^{14}C_6$]-NPE4 spiking standard (not shown), further indicating that no biodegradation occurred in the formaldehyde-spiked slurry bottles. The chromatographic NPEO distribution changed dramatically, however, in the oxic sediment slurry bottles during the course of the experiment compared with the corresponding controls (Fig. 3). All NPEO peaks were noticeably reduced in intensity relative to killed controls after 85 d, and this was particularly the case for NP1–4EO. By day 85, NP and the more highly ethoxylated NPEOs were more dominant contributors to the total NPEO mixture than at the beginning of the oxic slurry experiment, and this is reflected in Figure 2A. In contrast, the chromatographic NPEO distribution did not change dramatically in the anoxic sediment slurry bottles by day 91 (Fig. 3). In this case, all NPEO ethoxymer peaks were reduced in intensity relative to the anoxic killed control (Fig. 3), but the ethoxymer distribution was preserved relatively intact.

Formation and fate of NPEO degradation products in sediment slurry incubations

The HPLC radiochromatograms obtained from extracts of oxic and anoxic slurry bottles (Fig. 3) also revealed the presence of significant ^{14}C activity in late-eluting peaks. These peaks were not present in either oxic or anoxic control slurries (Fig. 3). The late-eluting peaks should correspond to compounds more polar than the NPEOs themselves, as they are more highly retained under the normal-phase HPLC conditions. The peak identities were confirmed as NP1EC, NP2EC, and NP3EC by retention-time match with characterized standards and by analysis of the collected fractions by ESI-MS/MS (Fig. 4). Under negative ionization conditions, NPECs may be selectively analyzed by electrospray mass spectrometry [16,25,26]. In the collision cell of a triple quadrupole mass spectrometer, NPECs undergo specific fragmentation to yield the negatively charged nonylphenolate ion, m/z = 219 (Fig. 4). This ion may be used as a diagnostic marker for NPECs in MS/MS analysis. The formation of NPECs as metabolites of NPEOs under aerobic conditions (Fig. 1) is well established [27–29], and it is not surprising that these compounds were detected as degradation products in the oxic sediment slurries of the present work. It is interesting, however, that NPECs, which are oxidation products of NPEOs, were detected as significant metabolites in the anoxic slurries (Fig. 3). Similar results have recently been reported by Schröder [23] for NPEO degradation in wastewater inocula under anaerobic conditions. These findings may help to explain the high concentrations of NPECs sometimes observed in anaerobically digested sewage sludge [30], and they indicate that some anaerobic microbial communities have the ability to oxidatively metabolize xenobiotics such as NPEOs in an anoxic environment.

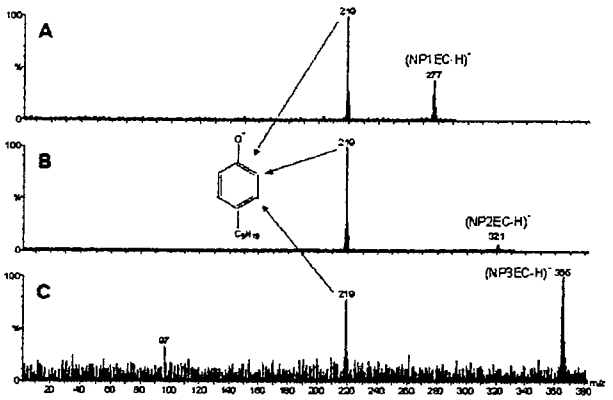

Fig. 4. Negative ion electrospray tandem mass spectra (ESI-MS/MS, 26 eV collision energy) of nonylphenol ethoxycarboxylates (NPEC) isolated from collected high-performance liquid chromatography (HPLC) fractions of an oxic sediment slurry extract. The proposed structure of the major diagnostic fragment ion is shown (nonylphenolate ion) for NP1EC (**A**), NP2EC (**B**), and NP3EC (**C**).

Levels of NPECs were found to increase most rapidly in the first 20 d of both the oxic and anoxic slurry bottle experiments (Fig. 2C and D). They then remained at a relatively constant level throughout the remainder of the incubations, eventually comprising 11 and 10% of the initially added ^{14}C activity in oxic and anoxic treatments, respectively, at the end of the experiment. Although the levels of NPECs were quantitatively similar in the oxic and anoxic sediment slurries, the NPEC mixture composition was quite different between these treatments. Oxic sediment slurries contained similar proportions of NP1EC, NP2EC, and NP3EC throughout the degradation experiment. Additional small chromatographic peaks were observed to elute after these compounds in the analysis of oxic slurry extracts (Fig. 3), and these peaks may have been

longer chain NPECs. Terminal ethoxy chain oxidation has previously been reported for long ethoxy chain NPEOs under aerobic conditions, leading to the formation of long-chain NPECs [16]. However, this could not be confirmed by ESI-MS/MS in the present work, as the suspect peaks gave insufficient signal in ESI-MS for tandem MS analysis. It is clear that, if these peaks were indeed NPE_nCs with $n > 3$, they did not contribute appreciably to the total NPEC pool. These additional peaks were also noticed at similarly low levels in anoxic slurry extracts (Fig. 3). Unlike in the oxic slurries, NPEC mixtures in the anoxic treatments were dominated by NP1EC until near the end of the experiment, at which point the NP1EC concentration declined to approximately the same level as NP2EC and NP3EC. In general, NP2EC has been reported as the most quantitatively important NPEC formed during aerobic NPEO degradation [16,29], although Ahel et al. [13] found NP1EC to be most abundant. Very few data are available for relative NPEC abundance after anaerobic degradation of NPEOs. Schröder [23] reported that NP2–3EC peaks were dominant in the atmospheric-pressure chemical ionization mass spectrometry spectra of anaerobically degraded NPEOs after 25 d.

Nonylphenol ethoxylates, including NP, have previously been reported to be converted to carbon dioxide (Fig. 1) under aerobic conditions in soil [31], activated sludge inoculum [32,33], and marine water and sediment [17], with typically between 40 and 60% of the test NPEO material mineralized over one to two month time scales. These previous results are in sharp contrast with the current findings, where only 1.7% of the initially added [$^{14}C_6$]-NPE4-derived radioactivity was present as CO_2 in the oxic treatment after 116 d of incubation (Fig. 2C). Similar results were obtained in the case of anoxic slurries, where the CO_2 pool accounted for only 2.3% of the initially added ^{14}C activity after 129 d (Fig. 2D). In contrast, relatively rapid (20% in 58 d) mineralization of ^{14}C-NP has previously been found to occur in anoxic marine sediment slurries [17]. It is possible that the low level of NPEO aromatic ring mineralization found in the current work was due to the use of alternative carbon sources by sediment microbes in the slurry bottles. The organic carbon content of the test sediment was rather high (near 8%), and therefore the microbial community likely was presented with a variety of substrates on which to subsist. The total CO_2 production in the oxic slurry bottles by the end of the experiment was approximately 2.0 mmol (calculated from the measured total oxygen consumption, assuming $CH_2O + O_2 \rightarrow CO_2 + H_2O$), well in excess of the total $^{14}CO_2$ produced from [$^{14}C_6$]-NPE4 (0.7 nmol, measured in the final oxic slurry bottle). It is likely that the methanol added along with the [$^{14}C_6$]-NPE4 spike at the beginning of the experiment served as a microbial carbon substrate during the course of the experiment; however, the amount added (0.247 mmol) could not have accounted for more than approximately 10% of the calculated total CO_2 production in the oxic experiment. It is thus probable that the microbial community utilized various carbon sources within the sediment. Nonylphenol ethoxylates were not likely to have been the most easily utilized carbon source in this complex mixture. On the other hand, the test sediment (from which the microbes presumably came) has been found to be highly contaminated with NPEOs [3,11], and therefore the resident microbial community should have already been readily acclimated to the use of NPEO as a substrate. Evidence for this preacclimation is provided by the lack of an observable lag period at the beginning of the NPEO decay curves (Fig. 2A and B). Another explanation for the relatively low level of NPEO mineralization found in the current study relative to previous work with NP in sediments [17] may be reduced bioavailability of NPEOs due to enhanced sorption to the highly organic-rich sediment used in the present work. Finally, microbial mineralization of NPEOs in the sediment slurries may have been inhibited by high concentrations of toxicants. Sediment from Grassy Bay has previously been reported to be highly contaminated with heavy metals and organic contaminants [19] and to be toxic to microorganisms in Microtox® assays [34].

Over the course of the biodegradation experiment, significant radioactivity was found to accumulate in the aqueous phase of the sediment slurry (Fig. 2C). This activity was not removed from the acidified aqueous solution by repeated liquid-liquid extraction with dichloromethane. At the end of the incubation, this metabolite pool was found to comprise 8% of the initially added ^{14}C activity in the oxic treatment (Fig. 2C). In contrast, only minimal radioactivity was observed in the aqueous phase of the anoxic sediment treatment during the degradation experiment (Fig. 2D). By the end of the anoxic incubation, the aqueous pool accounted for only 0.3% of the initially added ^{14}C activity. Based on this behavior, it was deemed likely that this uncharacterized pool of ^{14}C comprised highly oxidized acidic metabolites of NPEOs that were too water soluble to be efficiently removed from the aqueous solution by the dichloromethane extraction. Such metabolites, hypothesized to be dicarboxylated alkylphenol ethoxylates (CAPECs, Fig. 1), have previously been reported to occur and persist after aerobic degradation of NPEOs [6,16,29]. These compounds are formed from NPEOs by oxidation of the ethoxy chain and subsequent oxidation of the branched alkyl chain of the nonylphenol hydrophobe [29]. In the present work, it was not possible to confirm the presence of CAPECs in the extracted aqueous phase, as insufficient analytical signal was available for ESI-MS/MS analysis. However, the residual ^{14}C activity in the aqueous phase from the oxic treatment was found to be extracted quantitatively by graphitized carbon black (GCB; Carboprep 200; Restek, Bellefonte, PA, USA), and 50% of this activity was then eluted from the GCB with 50 mM formic acid in 80:20 dichloromethane:methanol. This behavior is consistent with that previously observed for CAPECs [6,29]. The remaining 50% of the ^{14}C activity extracted from the aqueous phase by the GCB was not recoverable in aqueous or solvent washes, nor was it found to remain adsorbed to the GCB material (no radioactivity was recovered from combustion of the GCB in the Harvey biological oxidizer, as described above for sediments). This activity remains unaccounted for but may have been in the form of low molecular weight acids (acetic, propionic, etc.) formed during NPEO metabolism that were extracted from the aqueous phase onto the GCB material, subsequently eluted, and lost to volatilization as the eluent was blown dry under a nitrogen stream. In any case, it seems likely that at least 50% of the ^{14}C activity remaining in the aqueous phase of the oxic sediment treatment after dichloromethane extraction was due to the presence of CAPECs. These NPEO metabolites have never been reported as degradation products under anaerobic conditions, and this is consistent with the lack of ^{14}C activity detected in the residual aqueous phase of the anoxic sediment slurries in the present work (Fig. 2D).

The balance of the ^{14}C activity initially added to the sediment slurries that was not accounted for by NPEOs or dis-

cernible metabolites was recovered as CO_2 after combustion of the extracted sediment residue. This pool of ^{14}C remained constant at approximately 3% of the initially added radioactivity in the anoxic sediment treatment throughout the degradation experiment (Fig. 2D). This suggests that the residual ^{14}C recovered from the extracted anoxic sediment by combustion was not modulated by the incubation and was therefore due to incomplete extraction of the spiked [$^{14}C_6$]-NPE4 material from the sediment during sample preparation. Further evidence of this was provided by the similar levels of ^{14}C activity (~3% of initial) recovered after combustion of residual sediment from anoxic killed control slurries. The low level of ^{14}C activity recovered from the residual sediment in this case (~3%) indicates that the NPEO extraction efficiency in sample preparation was not less than approximately 97%. Also, it can be concluded that no appreciable radioactivity was bound to the sediment matrix during the anoxic sediment incubation. This was not the case in the oxic sediment treatment. Nonextractable ^{14}C residual activity in the oxic sediment was observed to rise slowly from approximately 3% of initial activity at the beginning of the experiment until about day 60 of the experiment and then sharply to the end of the experiment, at which time this pool accounted for 27% of the initially added [$^{14}C_6$]-NPE4 spike (Fig. 2C). At the end of the oxic sediment slurry incubation period, this nonextractable ^{14}C associated with the sediment was the single largest pool of [$^{14}C_6$]-NPE4-derived radioactivity. There are several possible explanations for this result. The [$^{14}C_6$]-NPE4 may have been metabolized by bacteria and subsequently incorporated into microbial biomass, which would then have been poorly extracted by the solvents used in sample preparation. Alternatively, the radiolabeled compounds may have been covalently linked to the solid sediment substrate via biotic or abiotic processes. This behavior has previously been reported for phenols in soil organic matter [35]. Finally, the [$^{14}C_6$]-NPE4 material may have become sequestered in desorption-resistant organic or mineral phases in the sediment over the course of the degradation experiment [36]. This may have been due to alterations to the sediment mineralogy related to the observed changes in the slurry pH and E_h over time. However, the limited increase in sediment-bound ^{14}C activity in the formaldehyde-spiked oxic control slurry bottles (from 3 to 6.5% over the course of the experiment) indicates that the large majority of this increase observed in the oxic degradation treatment bottles was due to biotic processes, including incorporation of radiolabel into bacterial biomass and microbially mediated linking of NPEOs and metabolites to the sediment surface.

Kinetics of NPEO disappearance

The decay kinetics of individual NPEO ethoxymers as well as total NPEO were modeled in oxic and anoxic treatments by first-order decay as described by

$$C_t = C_0 e^{-kt}$$

where C_t is the concentration of a given NPEO ethoxymer at time t, C_0 is the initial concentration of this ethoxymer, and k is the first-order decay rate constant, with units of reciprocal time. For the model fit, measured NPEO decay curves were plotted as ln C_{NPEO} versus time, and the following equation was regressed against the data using a linear least squares algorithm (SigmaPlot 6.0, SPSS, Chicago, IL, USA):

$$\ln C_t = -kt + \ln C_0$$

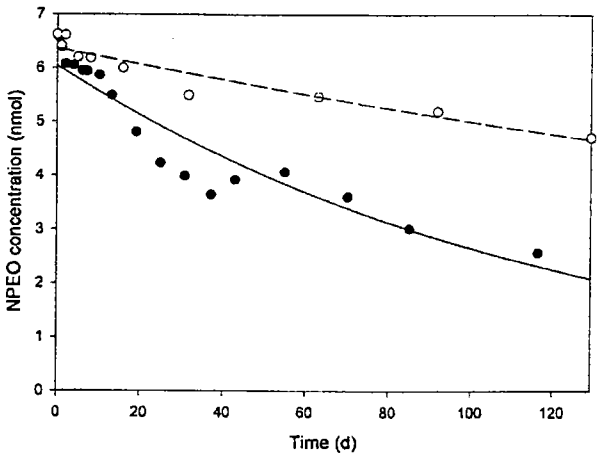

Fig. 5. Observed disappearance of total nonylphenol ethoxylates (NPEO) in oxic (●) and anoxic (○) sediment slurry bottle experiments, showing fit of first-order decay models (oxic: solid line, adjusted $r^2 = 0.89$; anoxic: dashed line, adjusted $r^2 = 0.90$).

This linear regression was used in lieu of an exponential fit because the natural logarithm transform tended to increase the normality of the fitted data. An example of the decay model output is shown in Figure 5 for total NPEO disappearance in oxic and anoxic sediment slurries. In this figure, the parameters (k and C_0) obtained from the fit after natural logarithm transformation were used to present the data and models on a linear scale. The significance of each regression was evaluated using an F test, and first-order decay rate constants are reported only for regressions that were significant at $p < 0.05$. In all cases where the regression was significant, k values obtained from the model fit were found to be statistically different from zero at $p < 0.05$ (Student's two-tailed t test). It must be noted that the F test for regression significance evaluates only whether the chosen model accounted for the majority of the variance observed in the fitted data. Goodness of fit in the model regression in this case does not imply that the NPEO ethoxymers or total NPEO mixture actually degraded via first-order kinetics. The assertion is that, in some cases in the present study, observed NPEO disappearance was relatively well described by first-order decay, and the distinction is made between the decay rate constant derived from the model fit to the observed disappearance and the degradation rate constant, which depends on an assumed degradation reaction order.

Fitted first-order decay rate constants, k, for individual NPEO ethoxymers as well as total NPEO in oxic and anoxic sediment slurry treatments are shown in Figure 6. Rate constants were typically low (<0.005/d) for all NPEO ethoxymers and the total NPEO mixture in the anoxic slurry treatments. No significant differences were detected among fitted decay rate constants for NPEO ethoxymers in the anoxic treatments (one-way analysis of variance, $p > 0.05$), indicating that all NPEOs decayed at very similar rates under anaerobic conditions in the present work regardless of ethoxy chain length. This is consistent with the observed preservation of NPEO ethoxymer distributions in anoxic sediment incubations, noted previously. No decay rate constant was determined for NP1EO in the anoxic slurry treatment, as the observed behavior of this compound was not consistent with first-order decay (discussed previously).

The total NPEO mixture decayed significantly faster in oxic sediment treatments ($t_{1/2}$ = 85 d, Table 1) than in the anoxic

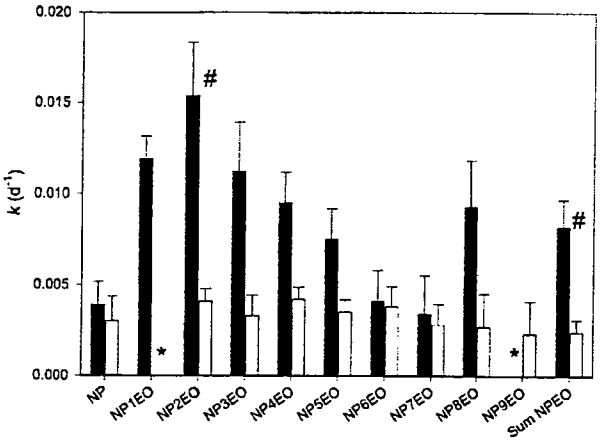

Fig. 6. First-order decay rate constants (±95% CI) for nonylphenol ethoxylates (NPEO) in oxic (shaded bars) and anoxic (open bars) sediment treatments. Asterisks (*) in place of bars indicate that the variance in measured concentration with time was not statistically well described by a first-order decay model (F test, $p > 0.05$). Pound symbols (#) indicate significant differences between anoxic and oxic first-order decay rate constants for individual NPEO ethoxymers and for sum NPEOs (Student's two-tailed t test, $p < 0.05$).

slurries ($t_{1/2}$ = 289 d, Table 1); however, for the individual NPEO ethoxymers, this difference was only statistically significant for NP2EO (Fig. 6). In contrast with the anoxic treatments, a one-way analysis of variance revealed that there were significant differences among the k's obtained for the individual NPEO ethoxymers in the oxic slurries ($p < 0.0001$). A subsequent multiple comparison test (Tukey's test) showed that pairwise differences existed between various k's of NPEO ethoxymers in the oxic treatments but that no adjacent pairs of NPEO ethoxymers (e.g., NP2EO/NP3EO or NP3EO/NP4EO) were significantly different with respect to decay rate constants at $p < 0.05$.

In general, NPEO K's were observed to increase from NP to NP2EO under aerobic conditions and then to decrease with increasing NPEO ethoxy chain length (Fig. 6). This trend may have been due to sorption-limited degradation of selected NPEOs in the oxic treatments. If NPEO degradation occurred chiefly in the aqueous phase of the sediment slurry, then the observed decay rates of individual NPEO ethoxymers would necessarily be reflective of the relative proportion of these compounds sorbed versus dissolved. A local minimum in sorption coefficients may exist for the NPEO ethoxymer series at or near NP2EO, as partitioning of these compounds to sediments seems to operate via two separate mechanisms. The more hydrophobic NPEOs (those with shorter ethoxy chains) more readily associate with organic phases of natural particles [11,12], whereas more highly ethoxylated molecules show greater affinity for sediment mineral phases, presumably due to specific interactions with the surface such as hydrogen bonding [12,37]. If a sorption minimum did exist for NPEOs with relatively short ethoxy chains, then these compounds would have been more readily partitioned into the dissolved phase in sediment slurries and hence more available for solution-phase degradation processes. The lack of NPEO ethoxymer-dependent decay rates in the anoxic slurries might then indicate that NPEO degradation in these treatments proceeded via sorption-independent pathways, i.e., in direct association with the solid phase. An alternative explanation may be that disappearance patterns (and thus apparent decay rates) of NPEO ethoxymers were complicated in anoxic treatments due to complex parent–metabolite relationships among the various ethoxymers in the NPEO mixture.

Nonylphenol ethoxylate decay kinetics observed in the present work can be compared with results previously reported for these compounds in various biodegradation tests (Table 1). Reported half-lives covered a wide range, from less than a day for NP in water to 60 years for NPEOs in bedded sediment (Table 1). It should be noted that the test conditions employed in the cited studies also varied considerably, and in some cases, the observed declines in NPEO concentration may have been due to processes other than degradation, such as sorption or volatilization. Nevertheless, some trends are evident in the data summarized in Table 1. Nonylphenol ethoxylates generally had shorter half-lives under oxic versus anoxic conditions, consistent with findings reported in the current study. Also, NPEO decay in solid media, such as sediment, soil, and sludge, was typically slower than that observed in aqueous solutions such as seawater, estuarine water, river water, or wastewater.

Nonylphenol ethoxylates in anoxic bedded sediments had the longest reported half-lives, in all cases greater than one year (Table 1). In contrast, half-lives of NPEOs in anoxic sediment slurries in the present work were in all cases less than one year. It is unlikely that differences between the physicochemical properties of bedded sediment and the sediment slurries contributed significantly to this discrepancy, as batch sediment slurry bottle experiments have previously been shown to be generally good predictors of bedded sediment initial degradation rates for relatively hydrophobic organic compounds in high solid to water ratio (0.01 g/ml and above) slurries, such as those used in the current study (~0.02 g/ml) [38]. Also, as noted previously, the pH, E_h, and dissolved oxygen were similar in the anoxic slurries and the freshly collected sediment. However, although the incubation temperature (25°C) was not greatly different from summertime bottom water temperatures recorded at the sediment collection site in Jamaica Bay, the water temperature at this site may fall to nearly 0°C during winter [18]. Because NPEO degradation rates in sediment are likely temperature dependent, the degradation of NPEOs in field sediments may proceed at considerably slower rates than reported in the current study during periods of colder water temperature.

Pathways of NPEO degradation in oxic and anoxic sediment slurries

One recently postulated mechanism by which NPEOs degrade under aerobic conditions suggests that the ethoxy chains of NPEOs are terminally oxidized to form long ethoxy chain NPECs, which then degrade to short-chain NPECs such as NP1EC and NP2EC, with formation of CAPECs by oxidation of the branched nonyl chain of NPECs [16]. In the present work, however, NPEOs seem to have degraded directly to short ethoxy chain NPECs, without noticeable accumulation of the longer chain NPEC intermediates in the oxic sediment treatments. Also, in contrast with the results reported by Jonkers et al. [16], some evidence for the formation of short-chain NPEOs (specifically NP3EO) as biodegradation intermediates was observed in the present work under aerobic conditions. This result is consistent with previous findings reported by Ahel et al. [4] and Kvestak and Ahel [14]. The presence of substantial [$^{14}C_6$]-NPE4-derived radioactivity in the residual aqueous phase of the oxic sediment slurries was consistent with the formation of CAPECs as significant NPEO metabo-

Table 1. Summary of NPEO degradation half-lives in environmental media

Experimental conditions	Half life (d) NP	NP1EO	NP2EO	NP3EO	NP4EO	NP5EO	NP6EO	NP7EO	NP8EO	NP9EO	Reference
Sediment slurry (oxic)	178	58	45	62	73	92	169	204	75		This study
	●———	———	———	———	85 (NP0–9EO)		———	———	———	———●	
Sediment slurry (anoxic)	231		169	210	165	198	182	248	257	301	This study
	●———	———	———	———	289 (NP0–9EO)		———	———	———	———●	
Bedded sediment (anoxic, *in situ*)	7,300	438									[20]
Bedded sediment (anoxic, *in situ*)	●———	———	———	———	22,000 (NP0–19)EO		———	———	———	———→	[9]
Sediment slurry (oxic)	69										[17]
Sediment slurry (anoxic)	161										
Seawater (oxic)	58										
Estuarine water (oxic, *in situ*)	●———	———	<30 (NP0–3EO)	———	●———						[11]
Estuarine water (oxic)		●———	———	———	2.5–69 (NP1–18EO)		———	———	———	———→	[14]
Estuarine water (oxic)								<24 (NP7–24EO)	———	———→	[15]
River water (oxic)					●———	———	0.42 (NP4–15EO)	———	———	———→	[16]
Mesocosm water (oxic)	0.34–1.2										[40]
Mesocosm sediment (oxic)	28–104										
Sewage sludge, municipal solid waste (anoxic)	15–>150 (NP0–2EO)										[22]
Sewage sludge, soil mixtures (oxic and anoxic)	3–>119[a]										[39]
Soil (oxic)	34.5–17										[31]
Activated sludge inoculum (oxic)	20										[33]
Activated sludge inoculum (oxic)		●———	———	———	———	28 (NP1–18EO)	———	———	———	———→	[32]
Activated sludge isolate (oxic)	2.4										[41]
Wastewater coliform isolate (oxic)			●———	———	———	17 (NP2–20EO)	———	———	———	———→	[42]
Shake-flask culture (oxic)		1.9–69 (NP1–2EO)									[13]

[a] Test conducted using *n*-NP.

lites in the present work. These compounds were most likely derived from oxidation of previously formed NPECs.

A final biologically mediated pathway, which has not been previously reported for NPEOs under aerobic conditions, seems to have influenced the disposition of [$^{14}C_6$]-NPE4-derived radioactivity in the oxic sediment slurries. A significant fraction of the initially added ^{14}C activity was strongly bound to the sediment at the end of the oxic slurry incubation. Thus, incorporation of carbon derived from the NPEO aromatic ring into microbial biomass and/or covalent binding of NPEOs and metabolites to sediment were important pathways in the observed degradation of the NPEO mixture within oxic sediment slurries. No evidence was observed for the formation of NP as a metabolite under aerobic conditions in the present work, consistent with the findings of Jonkers et al. [16].

The observed pathway of NPEO degradation in anoxic sediment slurries was relatively straightforward. Disappearance of NPEOs over the course of the anoxic experiment was accompanied by early formation of NPECs, as previously reported by Schröder [23]. Nonylphenol ethoxylates also appeared to degrade anaerobically by shortening of the ethoxy chain, as an increase in NP1EO was noted toward the end of the anoxic slurry incubation. Although formation of NP as a metabolite of NPEOs during anaerobic degradation was not detected over the time scale of the present experiment, this pathway of NPEO degradation may be important over longer time scales in bedded anoxic sediment [20]. In contrast with the results of the oxic slurry incubation, biotransformation of NPEOs to highly water-soluble metabolites such as CAPECs was not a major mechanism of degradation in anoxic slurries. Also, [$^{14}C_6$]-NPE4-derived radioactivity was not observed to become incorporated into the sediment matrix under anaerobic conditions. Finally, aromatic ring mineralization was apparently not a major pathway of NPEO degradation in either oxic or anoxic sediment slurries, as only minimal production of $^{14}CO_2$ was detected during over the course of the biodegradation experiments. This result is somewhat surprising, as NPEOs have previously been reported to be rapidly mineralized in a variety of environmental media [17,31–33,39], and suggests that NPEOs and their discrete metabolites are somewhat persistent under both oxic and anoxic conditions within at least the presently studied estuarine sediment from Jamaica Bay.

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