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Anaerobic Degradation of Nonylphenol Mono- and Diethoxylates in Digestor Sludge, Landfilled Municipal Solid Waste, and Landfilled Sludge

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The aim of this study was to investigate the extent to which anaerobic digestor sludge, landfilled sludge, and landfilled municipal solid waste (MSW) degrade NPEOs under methanogenic conditions. NPEO1 and NPEO2 (NPEO1-2), used in a mixture, were chosen as model compounds. Anaerobic experimental bottles were amended with 100% digestor sludge at three different concentrations of NPEO1-2: 2, 60, and 308 mg L^{-1}. [U-^{14}C]-NPEO1-2 was used to detect any possible decomposition of the aromatic moiety of the NPEO1-2. All inoculates used degraded NPEO1-2 at 2 mg L^{-1}, with nonylphenol (NP) forming the ultimate degradation product. The NP formed was not further degraded, and the incubations with labeled NPEO showed that the aromatic structure remained intact. Both landfill inoculates also transformed NPEO1-2 at 60 mg L^{-1}. CH_4 production was temporarily hampered in bottles with MSW landfill inoculum at 60 and 308 mg L^{-1}. With 2 mg L^{-1} of NPEO, CH_4 production closely followed that in the controls. Both NP and NPEO1-2 interacted with the organic matter which resulted in sorption to the solid phase.

Introduction

Nonylphenol ethoxylates (NPEOs) are extensively used as surfactants in industrial products. NPEOs are a mixture of polyethoxylated monoalkylphenols, predominantly para-substituted and are used in the manufacturing of paints, detergents, inks, and pesticides (*1*). Surfactants are common water pollutants because of their use in aqueous solutions, which are discharged into the environment in the form of wastewater from treatment plants or sludge stored in landfills. Degradation products of alkylphenol polyethoxylates, i.e., nonylphenol (NP), have the potential to be bioaccumulated, thereby becoming toxic to aquatic (*2*) and soil microorganisms (*3*).

The partial degradation of NPEOs can proceed both aerobically and anaerobically, and although the metabolic pathways are not completely understood, it is believed that biotransformation commences at the hydrophilic part of the molecule and that C-2 units (ethylene glycol) are removed one at a time (*4*), giving rise to nonylphenol mono- and diethoxylates (NPEO1-2). Complete degradation of NPEO1-2 may occur under aerobic conditions (*5*−*8*), but they have been reported to be more persistent in anaerobic environments (*9, 10*). Under aerobic conditions carboxylated metabolites may be formed (*8, 11*). Furthermore, because NPEOs with one or two ethoxy groups are less hydrophilic than polyethoxylated NPEOs, they are subjected to nonbiological elimination by sorption to hydrophobic sludge constituents and organic matter, among other materials.

The aim of this study was to qualitatively investigate the anaerobic biotransformation and degradation of nonylphenol mono- and diethoxylates by microorganisms derived from (i) an anaerobic sludge digestor treating wastewater from a pulp plant and industrial wastewater containg NPEOs as a pollutant, (ii) a landfill site where the very same sludge is deposited, and (iii) a municipal waste landfill, the latter acting as a reference source.

The use of these samples would give us an idea of if a microbial flora capable to degrade NPEO and NP had developed in the NPEO-contaminated digestor as well as if the deposited sludge is an environment allowing for NPEO and NP transformation. Finally, the MSW-sample was used as an inoculum, since landfill leachates have been shown to contain NP in the 10−100 μg L^{-1} range (*12*) indicating that there is a transformation capacity for NPEOs and/or NP-containing waste material in anoxic landfill environments.

Experimental Section

To investigate the potential for anaerobic microbial transformation of NPEO1-2, three inocula were used and incubated in 123 mL experimental bottles at 30 °C or 37 °C (see below). To each inoculum 2, 60, and 308 mg L^{-1} of NPEO1-2 and [U-^{14}C]-NPEO1-2 was added, respectively. Inoculated experimental bottles incubated with unlabeled NPEO1-2 at 2 and 60 mg L^{-1} were analyzed for methane and frozen periodically to determine the extent to which added compounds were transformed. Inoculated bottles with 308 mg L^{-1} of the test substances were only analyzed for methane. The gas phase of each inoculated bottle amended with [U-^{14}C]-NPEO1-2 was sampled every second week for analysis of $^{14}CH_4$ and $^{14}CO_2$. At the end of the experiment, inoculated bottles with labeled material were extracted and analyzed with regard to labeled recovery. Inoculated and autoclaved bottles were included in the study to account for any possible nonbiological transformation of ^{14}C-NPEO1-2. Note that inoculated bottles with labeled and unlabeled NPEO1-2 as well as inoculated controls will be referred to as experimental bottles.

Chemicals and Synthesis of [U-^{14}C]-NPEO. Unlabeled NPEO1-2 was supplied by Akzo Nobel Surface Chemistry (Stenungsund, Sweden). [ring-U-^{14}C]-phenol was bought from Sigma Radiochemicals (St. Louis, U.S.A.). InstaGel for $^{14}CO_2$ counting was bought from Canberra Packard AB (Uppsala, Sweden). Quickscint 501 (Zinsser) for $^{14}CH_4$ counting was purchased from Scintvaruhuset (Uppsala, Sweden). Cyclohexane (Pestiscan) was supplied by Lab-scan

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(Malmö, Sweden), sodium hydroxide (p.a.) by Akzo Nobel (Stockholm, Sweden), and pentafluorbenzoyl chloride (PFBCl) by Fluka AG (Buchs, Germany). Yeast extract (Oxoid) and the other chemicals (MERCK-products) used in the experiments were bought from KEBO (Stockholm, Sweden). Sodium sulfate was heated to 600 °C overnight, cooled, and stored in a desiccator before use.

^{14}C-Nonylphenol (^{14}C-NP; 1 mCi/mmol) was synthesized according to Ekelund et al. (*5*). The ethoxylation of ^{14}C-NP was done in the following way: 0.20 g (0.90 mmol, 1 mCi mmol^{-1}) of ^{14}C-NP and 0.24 mL 0.53% w/w (0.024 mmol) of sodium methoxide (dissolved in methanol) were added to a Pierce Reacti-Thern Heating/Stirring module No. 18971 under a nitrogen atmosphere. The methanol was evaporated under a stream of nitrogen for 80 min at 60 °C. The vial was cooled to 4 °C whereupon 1.12 mL of a 71 mg mL^{-1} solution of ethylene oxide (EO; 1.80 mmol) in toluene was added in a glovebox with a nitrogen atmosphere. The vial was then heated to 140 °C for 18 h, whereafter the toluene was evaporated under a stream of nitrogen for 60 min at 140 °C. Weighing the sample showed that 0.046 g (1.04 mmol) of the EO added had reacted. To increase the amount of ethoxy groups, 0.24 mL of sodium methoxide was added, followed by 0.50 mL of the EO in toluene solution. After the second ethoxylation, a total of 0.079 g (1.80 mmol) of EO had reacted. The ^{14}C-NPEO1-2 synthesized was diluted with 2.49 g of methanol. The concentration of radioactive NPEO in the solution was determined to be 0.35 mCi g^{-1}. The synthesis procedure described above was carried out four times in total. Since three of the four batches synthesized were almost identical, they were mixed together and used in the anaerobic degradation investigation. The commercial NPEO1-2 and ^{14}C-NPEO1-2 synthesized have the following CAS number: 127087-87-0, 4-nonylphenol, branched, ethoxylated.

Inocula, Preparation, and Inoculation. The first inoculum was taken from a 15 000 m^3 mesophilic (37 °C) anaerobic digestor treating wastewater from a pulp plant. The digestor was also fed with a wastewater contaminated with NPEOs at ca. 60 mg L^{-1} during one week at six occasions per year. The periodic addition of NPEO contaminated wastewater gave rise to a final concentration of NPEO of approximately 2 mg L^{-1} in the digestor a conditions which had occurred for at least four years at the time for sludge sampling. The sludge residence time varied between 50 and 80 days depending on the amount of wastewater generated at the time being. Digestor sludge (10 L) was collected and stored for 20 h in a polyethylene can until inoculation took place (see below). The second inoculum originated from a landfill where the dewatered digestor sludge from the above-described treatment plant had been deposited. Three separate cores taken 20−20 m apart from each other were withdrawn with a hand drill from a depth of 1.5 m below the surface. At the time of sampling the landfill was approximately 3 m deep, and the temperature in the landfill was 15 °C. Two hundred g from each of the three cores was later on mixed together in order to generate a pooled sample. The third inoculum originated from a municipal solid waste landfill (Filborna Landfill, Helsingborg, Sweden). Solid waste samples (1−2 kg) were taken from a 100 kg sample originating from a depth of 16 m while drilling wells for landfill gas extraction. The municipal solid waste had been buried for approximately 20 years. Solid samples from the two landfills were further treated by diluting subsamples (200 g) according to Ejlertsson et al. (*13*).

A mineral medium (pH = 7.0) was prepared as originally described by Zehnder et al. (*14*) and modified by Ejlertsson et al. (*13*). The medium was made up from three stock solutions: the phosphate buffer with resazurin, the salt, trace element and vitamin solution (C1) and C2, a solution containing bicarbonate, yeast extract, and sulfide. A 40-mL portion of each the media was added to each of the experimental bottles (123 mL serum bottles) under a continuous flow of N_2. The bottles were closed with ethylene-propylene-dimer (EPDM) rubber stoppers and sealed with aluminum screw caps. The gas phase was exchanged for N_2/CO_2 (80:20), and the bottles were autoclaved at 121 °C for 30 min. During inoculation, a 10-mL automatic pipet was used to add 2.5 mL of C1 followed by 5 mL of one of the two landfill inocula or the digestor sludge, while flushing with N_2/CO_2 (80:20). In addition, 50 mL of sludge from the digestor was added to empty autoclaved bottles (123 mL serum bottles) with a N_2/CO_2 (80:20) atmosphere using a MasterFlex automatic dispenser (Cole-Parmer Instrument Co., Niles, U.S.A.). NPEO1-2 dissolved in three methanol solutions was added to the experimental bottles directly after inoculation to give final concentrations of 2, 60, and 308 mg l^{-1}. Experimental bottles that did not receive any NPEO1-2 (controls) were supplied with methanol corresponding to the amount used for the NPEO1-2 additions. The 100% and 10% digestor sludge incubations were made in triplicate and placed in the dark at 37 °C. Incubations with the two landfill inocula were made in triplicate and placed in the dark at 30 °C. The content of total solids (TS) in the experimental bottles after inoculation was 53 and 5.3 g L^{-1} for the 100% and 10% digestor sludge, respectively. The TS content was 2.0 and 1.3 g L^{-1}, respectively, for experimental bottles inoculated with landfilled digestor sludge and MSW.

For the labeled experiments, 0.32 μCi ^{14}C-NPEO1-2 dissolved in methanol was added to each bottle incubated with 2 mg L^{-1}. To the experimental bottles with 60 and 308 mg L^{-1}, 4.87 μCi (= 30 mg L^{-1}) was added, followed by 30 and 278 mg L^{-1} unlabeled NPEO1-2, respectively. In this way, a 1% degradation of the added labeled compounds would give rise to readily measurable amounts of $^{14}CH_4$ and $^{14}CO_2$. Bottles that had not received any ^{14}C-NPEO1-2 (controls) were supplied with methanol corresponding to the amount used for the ^{14}C-NPEO1-2 additions. Six bottles with ^{14}C-NPEO1-2 were prepared for each respective inoculum and concentration investigated. To account for abiotic degradation, two sets of triplicate bottles were autoclaved and incubated. Experimental bottles with ^{14}C-NPEO1-2 were incubated in the same way as the unlabeled bottles (see above).

Sampling and Analytical Procedures. *Labeled Incubations.* The six experimental bottles incubated for each respective inoculum and concentration of ^{14}C-NPEO1-2 were divided into three sets. One of the sets, i.e., two bottles for each inoculum and concentration used, was sampled every second week for $^{14}CO_2$ and $^{14}CH_4$ according to Schnürer et al. (*15*). At the end of the investigation, the experimental bottles were extracted with diethyl ether (see below). Portions (50-μL) of extract from experimental bottles with 60 and 308 mg L^{-1} were then transferred to scintillation vials containing 1 mL of methanol, whereupon 8 mL of InstaGel was added and the vials were closed with a rubber/Teflon-lined screw cap. A 500-μL amount of ether extract from each experimental bottle incubated at 2 mg L^{-1} was treated in the same way. Radioactivity in solid particles was determined as described by Schnürer et al. (*15*). Radioactivity was counted in a Beckman LS-1801 scintillation spectrophotometer (Beckman Instruments Inc., Fullerton, U.S.A.).

Unlabeled Incubations. Gas samples (0.3 mL) were withdrawn from the experimental bottles, and their methane content was quantified by gas chromatography as described by Ejlertsson et al. (*16*). Overpressure in the bottles was accounted for.

Experimental bottles with NPEO1-2 at 60 and 308 mg L^{-1} frozen during the experimental period were later thawed and extracted by adding 5 g of NaCl and 30 mL of diethyl ether followed by vigorous shaking for 4 min, so as to extract both the sludge and inside glass surface of the bottle. Di(2-

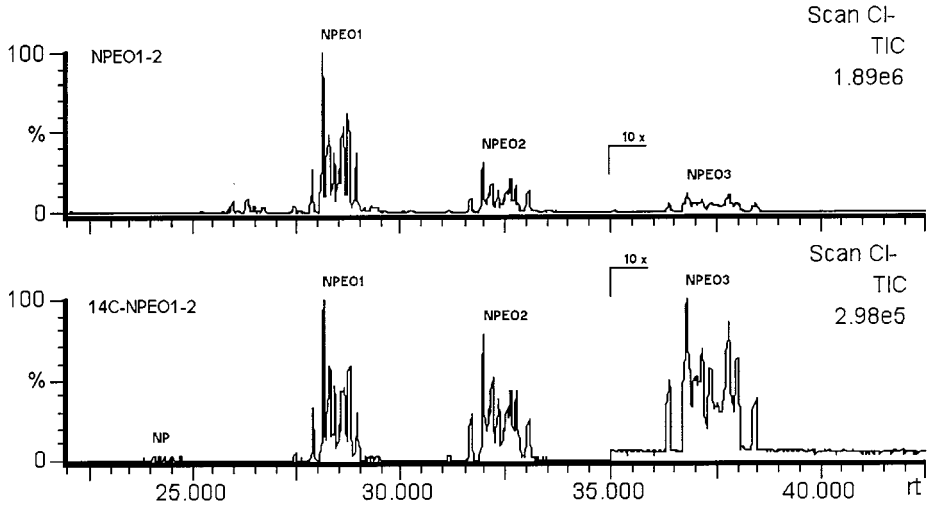

FIGURE 1. Chromatogram showing the proportional distributionsof NP and NPEO1-2 in the commercial unlabeled NPEO1-2 and in the ^{14}C-NPEO1-2.

ethylhexyl) adipate (DEHA) was used as an internal standard. A 2-μL amount of ether extract from the 308 mg L^{-1} incubations was injected manually in the split mode (15 mL min^{-1}) on a HP 5880 gas chromatograph equipped with a flame ionization detector (Hewlet-Packard, Kista, Sweden), using a BPX column (30 m × 0.32 mm, df. 0.25 μm; Scantec Lab, Partille, Sweden). Helium was used as carrier gas (2 mL min^{-1}), and detector and injector temperatures were set at 250 °C, respectively. Temperature programming was used to separate the compounds studied: 170 °C for 10 min, rise at 10 °C min^{-1} to 200 °C, and finally a rise at 5 °C min^{-1} to 300 °C. Ether extracts from experimental bottles with 60 mg of NPEO1-2 L^{-1} had to be concentrated 10-fold prior to the injection.

Experimental bottles with 2 mg of NPEO1-2 L^{-1} as well as experimental control bottles frozen during the experimental period were analyzed with the following analytical technique in order to be able to detect the low concentration used (2 mg L^{-1}) as well as to estimate the background content of NPEO1-2 and NP in each inoculum: The bottles were thawed and acidified with hydrochloric acid (3 M) to pH 2−3 followed by the addition of 3 mL of saturated sodium chloride solution. The pH was checked with indicator paper. After acidification the samples were extracted with cyclohexane (2 × 25 mL) in a separatory funnel. To break the emulsion that formed during the shaking in the separatory funnel, the organic phase was transferred to centrifuge tubes and treated in a sonication bath for 15 min and then centrifuged at 2500 rpm for 5 min. The water phase was transferred back to the separatory funnel and extracted once again. The two cyclohexane extracts were combined and dried with sodium sulfate, and the volume was reduced in a rotary evaporator to approximately 1 mL. The extract was then transferred to a 5-mL graded tube, and the volume was adjusted to 2 mL with cyclohexane. Standard solutions of the studied NPEO1-2, ranging in concentration from 0.077 to 9.5 μg mL^{-1}, were prepared in cyclohexane. Standard solutions of NP were prepared in concentrations ranging from 0.079 to 19 μg mL^{-1}. Standards were used to check the linearity as well as to determine the proportional distribution of the homologues in the NPEO1-2 mixture. Standards and extracts were derivatizated with pentafluorbenzoyl chloride (PFBCl) essentially according to the method described by Wahlberg et al. (*17*): The cyclohexane extracts (2 mL) were transferred to test tubes with Teflon-lined screw caps followed by the addition of PFBCl (10 μL) and pyridine (5 μL). The reaction mixture was kept at 60 °C for 15 min in a water bath, after which 10 mL of sodium hydroxide (1 M) was added and the test tube shaken for 1 min. After separation, 1 mL of the organic phase was collected in a 5-mL graded test tube and reduced to 0.5 mL before the gas chromatographic determination. The analyses were performed on a HP 5890 Series II gas chromatograph (Kista, Sweden) equipped with a DB-5 (60 m × 0.25 mm, df. 0.25 μm; J&W Scientific, Folsom, U.S.A.) using helium as carrier gas. A TRIO-1 mass spectrometer (VG Masslab, Manchester, GB) in the negative chemical ionization mode with methane as reactant gas was used as detector. The samples (2 μL) were injected in splitless mode at 250 °C injection temperature. The temperature program was 90 °C (1 min), 10 °C min^{-1} to 200 °C, 6 °C min^{-1} to 300 °C and 300 °C (16 min). Chromatograms were registered using selective ion monitoring of the molecular ions of the target compounds (m/z 414 for NP, m/z 458 for NPEO1, and m/z 502 for NPEO2). For m/z 458 and m/z 502 the whole area of the cluster of peaks was used for calculations. For m/z 414 the last peak of the cluster was excluded in the calculations, due to the presence of an interfering peak originating from both the digestor sludge and the landfilled digestor sludge. The relative amounts of NP, NPEO1, and NPEO2 in the studied NPEO1-2 mixture were determined with the GC/MS procedure described above. The peak in the m/z 414 cluster which was excluded in the calculations was small, and its exclusion did not significantly affect the resulting values. The extracts from the samples spiked with NPEO1-2 were diluted (15−150 times) prior to injection on the GC. The linearity of the MS-detection was found to be satisfactory within the concentration interval corresponding to the levels of NP, NPEO1, and NPEO2 in the samples.

Results and Discussion

The proportional distribution of NP, NPEO1, and NPEO2 in the commercial NPEO1-2 mixture studied was found to be about 0.15% NP, 70% NPEO1, 28% NPEO2, and 2% NPEO3 (Figure 1). The proportional distribution of the ethoxy groups from the ^{14}C-labeled NPEO1-2 synthesized and used for the labeled incubations was similar to the commercial NPEO1-2 (Figure 1).

The background content of indigenous NP and NPEO1-2 was high in the inocula used (Table 1), especially that of NP in the landfilled MSW. The proportional distribution of the homologues in the background differed from that found in the studied NPEO1-2, and none of the homologues in the background were transformed during the experiment. The background was subtracted to avoid disturbing the evaluation of the transformation of the spiked nonylphenol ethoxylate

TABLE 1. Background Levels of NP, NPEO1, and NPEO2 in Landfilled Digestor Sludge and Municipal Solid Waste and in 100% and 10% Digestor Sludge[a]

	(mg kg^{-1} dw)		
inoculum	NP	NPEO1	NPEO2
landfilled sludge	15.5 (10.5)	13.5 (17.5)	8.0 (17.0)
landfilled MSW	485 (4.2)	11.5 (16.2)	0.6 (29.2)
digestor sludge (100%)	0.7 (0.7)	0.9 (0.7)	0.04 (1.0)
digestor sludge (10%)	2.1 (4.9)	2.3 (10.6)	0.7 (17.0)

[a] The numbers represent the mean of the background levels measured in control bottles from all sampling occasions expressed as mg NP, NPEO1, or NPEO2 per kg of dry weight. The relative standard deviation is expressed within parentheses. dw = dry weight.

mixture. The relatively high concentration of NP in the landfilled MSW was most likely a result of the previously extensive use of 4-nonylphenol polyethoxylates as nonionic surfactants in industrial and household detergents. The occurrence of high NP concentrations is not surprising, since they are in the same orders of magnitude as the concentrations observed for NP (<10−107 μg L^{-1}) in landfill leachate (*12*). Assuming that NP is adsorbed on the solids of the leachate and the TS content of mature landfill leachate is in the range of 0.5 to 5 g L^{-1}, the concentration of NP will be in the range of <2−214 mg NP per kg solids of leachate.

At a concentration of 2 mg NPEO1-2 L^{-1}, anaerobic microorganisms in all inoculates included in this study transformed parts of added NPEO1-2 to NP. The highest degree of transformation occurred with the two landfill inocula. The background content of NP in bottles inoculated with landfilled MSW was so high that a transformation of 2 mg NPEO1-2 L^{-1} would only increase the indigenous NP concentrations with 5−10%. Thus, the absolute concentrations of NPEO1 and NPEO2 in bottles amended with 2 mg NPEO1-2 L^{-1} went down significantly within 22 days of incubation (Figure 2A), while only small changes in the amount of the background NP was observed (data not shown). In bottles containing the landfilled digestor sludge the relative proportion of NP increased from 0.15% to 81% during 53 days of incubation, whereafter the proportional distribution of the homologues remained constant (Figure 2B). Transformation of NPEO1-2 was also observed at 60 mg L^{-1} for both landfill inoculates (Figure 3A−B). The landfill for the anaerobic digestor sludge was continually amended with sludge from the anaerobic digestor which should have mainly contained transformed nonylphenol polyethoxylates with shorter ethoxy chains. One explanation for the high transformation efficiency may be that the microorganisms in the sludge landfill had adapted to the short-chain nonylphenol polyethoxylates. For the landfilled MSW the same reasoning could be applied owing to the previously extensive use of 4-nonylphenol polyethoxylates as nonionic surfactants in industrial and household detergents. In the samples with 10 and 100% anaerobic digestor sludge amended with 2 mg L^{-1}, NP levels increased steadily during the experiment, at the end of which the NP level was 57% in the 100% digestor sludge sample and 31% in the 10% sludge sample (Figure 2C and D). Transformation of NPEO1-2 also occurred in samples amended with 60 mg L^{-1} for 100% sludge (Figure 3D), in which NPEO2 was transformed to NPEO1. However, no such transformation occurred in the diluted sludge inoculum (Figure 3C). At 308 mg L^{-1} less than 1% of the added NPEO1-2 was transformed into NP for the undiluted sludge and the landfilled sludge inoculum. Transformation of NPEO1-2 and/or formation of NP was not observed in autoclaved incubations.

No $^{14}CO_2$ or $^{14}CH_4$ could be detected in any of the experimental bottles to which labeled NPEO1-2 had been

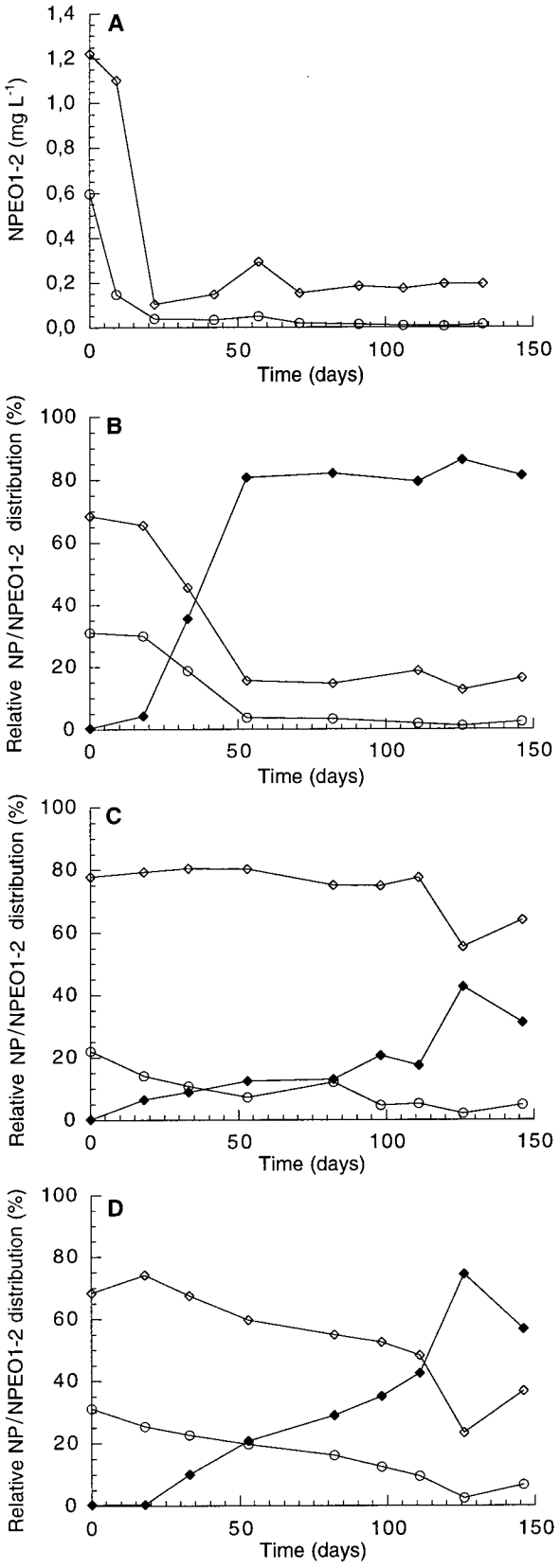

FIGURE 2. Absolute amounts of NPEO1 (◇) and NPEO2 (○) in experimental bottles inoculated with landfilled MSW at 2 mg of NPEO1-2 L^{-1} (A). Relative amounts of unlabeled NP (◆), NPEO1 (◇), and NPEO2 (○) in experimental bottles at 2 mg of NPEO1-2 L^{-1} inoculated with B = landfilled sludge, C = 10% digestor sludge, and D = 100% digestor sludge. Each mark on the curves represents an experimental bottle that was frozen during the experimental period and analyzed at the end of the study.

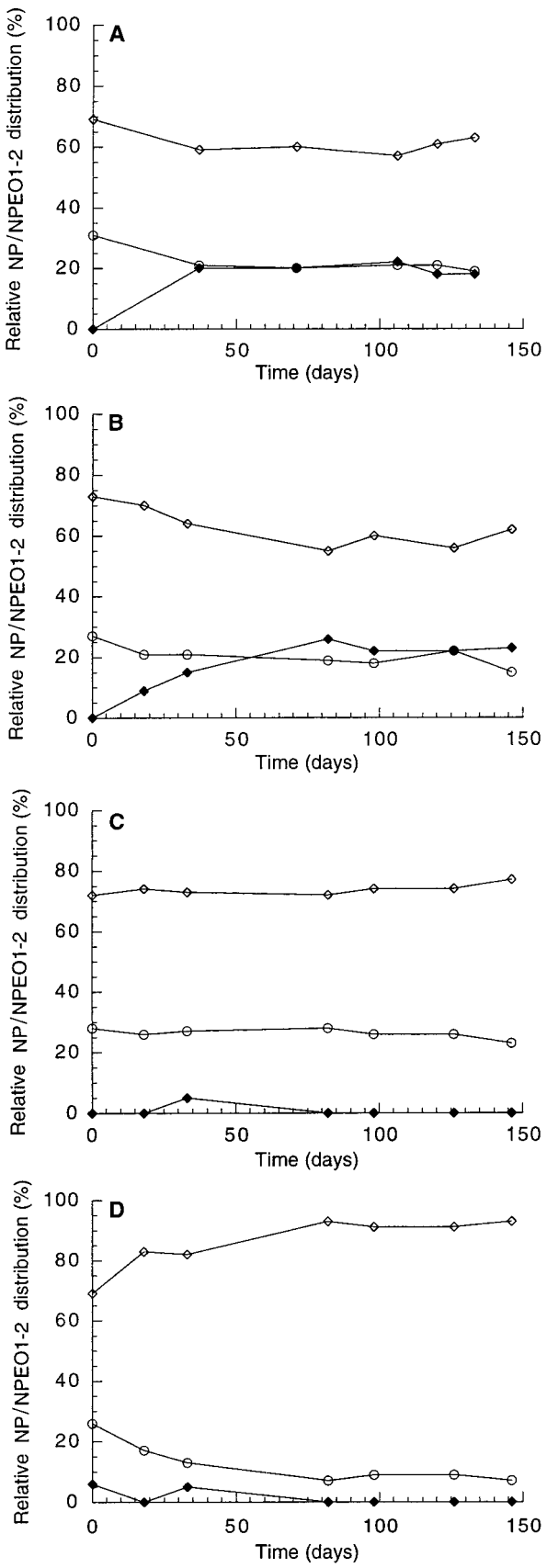

FIGURE 3. Relative amounts of unlabeled NP (◆), NPEO1 (◇) and NPEO2 (○) in experimental bottles at 60 mg of NPEO1-2 L^{-1} inoculated with A = landfilled MSW, B = landfilled sludge, C = 10% digestor sludge, and D = 100% digestor sludge. Each mark on the curves represents an experimental bottle that was frozen during the experimental period and analyzed at the end of the study.

TABLE 2. Recovery of ^{14}C-Labeled Material in Diethyl Ether (Extract), Solids and Culture Liquid after Extraction of Bottles with the Three Inocula at the End of the Experiment[a]

	(10^3 DPM)			
inocula	extracts	solids	liquid	recovery (%)
100% sludge				
2 mg L^{-1} autoclaved	530	190	19	104
2 mg L^{-1}	205	396	54	92
60 mg L^{-1} autoclaved	7580	1470	91	85
60 mg L^{-1}	3563	4567	167	78
308 mg L^{-1} autoclaved	8545	1776	136	98
308 mg L^{-1}	4957	3713	98	83
10% Sludge				
2 mg L^{-1} autoclaved	529	11	22	79
2 mg L^{-1}	430	12	24	65
60 mg L^{-1} autoclaved	9908	888	182	103
60 mg L^{-1}	8144	1424	176	91
308 mg L^{-1} autoclaved	10 430	865	150	107
308 mg L^{-1}	9985	1119	107	105
Landfilled Sludge				
2 mg L^{-1} autoclaved	509	93	10	86
2 mg L^{-1}	494	128	10	88
60 mg L^{-1} autoclaved	8519	1174	58	91
60 mg L^{-1}	8819	1409	41	96
308 mg L^{-1} autoclaved	9761	1740	69	108
308 mg L^{-1}	10 671	703	20	108
MSW				
2 mg L^{-1} autoclaved	565	57	6	88
2 mg L^{-1}	566	68	13	91
60 mg L^{-1} autoclaved	9567	717	42	96
60 mg L^{-1}	10 067	1046	46	104
308 mg L^{-1} autoclaved	10 306	569	33	102
308 mg L^{-1}	10 727	512	29	105

[a] The recovery was calculated based on the theoretical amounts of ^{14}C-labeled NPEO added (2 mg/L: 713900 DPM; 60 and 308 mg/L: 10707680 DPM).

added. The recovery of ^{14}C-labeled material found in diethyl ether extract, solid material, and the remaining liquid-phase amounted to at least 65% (mean recovery 94%; Table 2). The fact that almost all of the labeled material was found in the organic solvent phase and on the solid particles indicates that the decomposition of the NPEO1-2 mixture added most likely did not involve cleavage and further breakdown of the aromatic structure. In the undiluted sludge incubations, more than 50% of each of the labeled compounds was found in the solid phase after extraction with diethyl ether compared with 10−15% in the 10% sludge and the two landfill inoculates (Table 2). At 2 and 60 mg L^{-1} ^{14}C-NPEO1-2, autoclavation caused a decrease in the partitioning of labeled material among the solids in the sludge inoculates as compared with bottles that had not been autoclaved (Table 2). It should be noted that the sludge matrix may have changed during the experimental period, as indicated by the finding that concentrations of unlabeled NPEO1-2 decreased when extracted with cyclohexane (data not shown). The fact that a substantial fraction of the labeled material occurred in the solid phase of some inocula may have implications for the degradation processes: Sorption of the compounds to the solids decreases the susceptibility of these short-chained, hydrophobic compounds to microbial attack, thus promoting their persistence in contaminated systems. It has been reported earlier that the mass flow of short-chained NPEOs in sewage treatment plants as well as in natural aquatic environments depends not only on microbial activity but also on physiochemical processes (*6*, *18*).

Factors influencing the degradability of alkyl ethoxylates include the structure of their hydrophobic region, the length of their hydrophilic unit, and the incorporation of other glycol

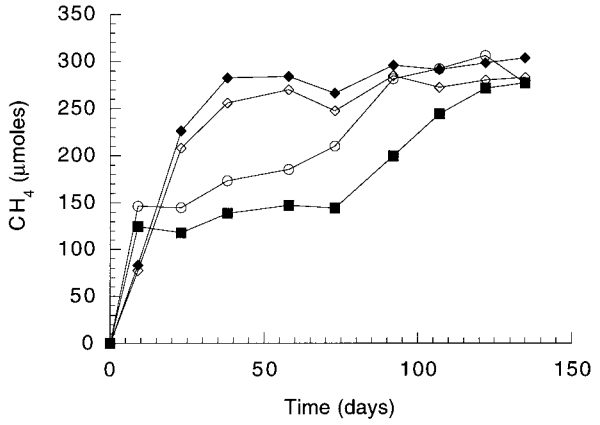

FIGURE 4. Formation of methane in bottles inoculated with landfilled MSW. Symbols represent methane formed in control bottle (◆), 2 mg of NPEO1-2 L^{-1} (◇), 60 mg of NPEO1-2 L^{-1} (○) and 308 mg of NPEO1-2 L^{-1} (■).

molecules in the hydrophilic region (*19*). Our observations on the degradation of NPEO1-2 are in line with earlier reports (*9, 10*) showing that under anaerobic conditions NPEOs can be degraded to NP, which is far more persistent than its parental compounds. Surfactants containing straight-chain alcohol ethoxylates can be transformed quickly and completely under anaerobic conditions, whereas branched alcohol ethoxylates often are more difficult for the organisms to degrade (*20*). Information on the anaerobic degradation of alcohol ethoxylates is limited: Salanitro and Diaz (*21*) showed, for example, that straight-chain alcohol ethoxylate LA-C_{9-11}+8EO gave a methane yield that was 84% of the theoretically calculated methane value. The concentration of the substance was initially 50 mgC L^{-1}, and the inoculum was milled and diluted municipal waste. However, the substance inhibited methane production during the first 30 days. In 1988, Wagener and Schink (*22*) showed that the linear-chain alkyl ethoxylates LA-C_{12}+4EO, LA-C_{12}+23EO, and LA-C_{16}+20EO were transformed completely to methane and carbon dioxide in enrichment cultures inoculated with anoxic sewage sludge. The concentration of surfactant was 1 g L^{-1}. It was found that long-chain fatty acids were released as intermediates.

The addition of NPEO1-2 did not result in an increase in the total amount of methane formed in any of the samples, suggesting that the phenol ring structure remained intact during the period of incubation. It is conceivable, however, that the EO-groups liberated as ethylene glycol during the degradation of higher NPEOs could have been further degraded to CH_4 and CO_2. However, the amount of EOs released during the transformation of NPEO1-2 to NP would have been too low to generate enough methane to result in levels substantially higher than those produced from the methanol added and the indigenous substrates of the inocula. The methanol added was most likely completely degraded by all inocula studied and accounted for approximately 190 µmol of the methane produced in experimental bottles with unlabeled NPEO1-2. In experimental bottles with labeled NPEO1-2, 660 µmol of methane formed via methanol degradation. At all NPEO1-2 concentrations studied, the temporal pattern of methane production in experimental bottles inoculated with 100% or 10% digestor sludge resembled that in the respective control. The formation of methane was intense during the first month of incubation, after which it gradually leveled off. For experimental bottles inoculated with landfilled MSW, the pattern of CH_4 production was different (Figure 4). At 308 mg L^{-1} NPEO1-2, CH_4 production equaled that in the controls for the first couple of weeks, whereafter it was inhibited. After ca. 80 days, CH_4 production was resumed, and at the end of the experiment amounts of methane were equal to those found in the controls (approximately 300 µmol CH_4). Methane production was also inhibited in samples amended with 60 mg L^{-1} of NPEO1-2, although to a lesser extent, whereas the pattern of production in samples amended with 2 mg L^{-1} of NPEO1-2 closely resembled that in the controls (Figure 4). Similar results were obtained with the landfilled digestor sludge at 308 mg L^{-1} of NPEO1-2. Although we cannot explain this response, it seems probable that NPEO1-2 or NP adversely affected the acetate-utilizing methanogens, thereby partially hampering methane formation. Battersby and Wilson (*23*) observed inhibitory effects of NP at 50 mgC L^{-1} on methane formation in a survey of the anaerobic biodegradation potential of organic chemicals in digesting sludge. Taking the observation made by (*23*) into consideration, the inhibitory effect on methanogenesis observed for the landfilled MSW inoculum may be caused by the formation of NP from NPEO1-2.

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