Mechanism for Biotransformation of Nonylphenol Polyethoxylates to Xenoestrogens in *Pseudomonas putida*  

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A strain of *Pseudomonas putida* isolated from activated sewage grew aerobically on the xenoestrogen precursor, nonylphenol polyethoxylate (NPEO*$_x$*, where *x* is the number of ethoxylate units) as sole carbon source. Comparative growth yields on NPEO$_{av4}$, NPEO$_{av7}$, and NPEO$_{av20}$ (mixtures with average ethoxylate numbers as indicated) were consistent with utilization of all but two ethoxylate units, and the final accumulating metabolite was identified by gas chromatography-mass spectroscopy as nonylphenol diethoxylate (NPEO$_2$). There was no growth on nonylphenol or polyethylene glycols, and there was no evidence for production of carboxylic acid analogs of NPEO$_x$. Biodegradation kinetics measured by high-pressure liquid chromatography (HPLC) for each component in NPEO$_x$ mixtures showed that biodegradation proceeded via successive exocision of the ethoxylate chain and not by direct scission between the second and third ethoxylate residues. The NPEO$_x$-degrading activity was inducible by substrate, and cell extracts of NPEO$_{av9}$-induced cells were also active on the pure alcohol ethoxylate, dodecyl octaethoxylate (AEO$_8$), producing sequentially, under either aerobic or anaerobic conditions, AEO$_7$, AEO$_6$, AEO$_5$, etc., thus demonstrating that the pathway involved removal of single ethoxylate units. HPLC analysis of 2,4-dinitrophenylhydrazone derivatives revealed acetaldehyde (ethanal) as the sole aldehyde product from either NPEO$_{av9}$ or AEO$_8$ under either aerobic or anaerobic conditions. We propose a mechanism for biotransformation which involves an oxygen-independent hydroxyl shift from the terminal to the penultimate carbon of the terminal ethoxylate unit of NPEO$_x$ and dissociation of the resulting hemiacetal to release acetaldehyde and the next-lower homolog, NPEO$_{x-1}$, which then undergoes further cycles of the same reaction until *x* = 2.

There is growing concern that the perceived reductions in the human male sperm count in the second half of this century, the increased incidence of testicular cancer (5, 32, 35), and the feminization of species inhabiting aquatic freshwater environments (30), may be attributable to endocrine-disrupting chemical pollutants in the environment. In addition to the identified need for studies on epidemiology and for toxicity-testing strategies and methods (6, 27), environmental risk assessments will be required for products from which endocrine disrupters (xenoestrogens) may be derived. Among the sources of putative xenoestrogens are the nonylphenol polyethoxylates (NPEO [see below]) (Fig. 1), which are commercially important surfactants with industrial, agricultural, and domestic applications. They are comprised of a phenol nucleus that is *o*, *m*, or *p* substituted with one of a variety of hydrophobic, branched, isomeric nonyl moieties, and with a hydrophilic polyethylene glycol (ethoxylate) chain either linked at the phenolic oxygen. As a result of the mode of synthesis from ethylene oxide monomers, the ethoxylate chains in commercial preparations are polydisperse, with lengths ranging from 1 to typically 50 or more ethylene glycol units; this variation is reflected in the designation NPEO$_x$ where *x* is the number of ethylene glycol (EO) units in the molecule and NP stands for nonylphenol. The combined annual production for the United States, western Europe, and Japan is estimated at about 0.35 Mt, and thus these compounds are common constituents of municipal wastewaters (2). While the higher homologs of NPEO lack estrogenic activity, the shorter NPEO$_x$ homologs and nonylphenol itself are known to exert estrogenic effects in aquatic organisms (18, 19, 39) and in mammals and birds (10, 33, 39).

Primary biodegradation of NPEO, in wastewater treatment plants is relatively efficient, but the resulting intermediates are more recalcitrant and ultimate degradation efficiency is significantly lower (2), so that degradation products, predominantly nonylphenol and lower homologs of NPEO$_x$, are found in sewage treatment effluents (11, 14, 17), rivers (3, 14), estuaries (21, 22), and coastal waters (25). Thus, the biodegradation process converts the relatively benign surfactants into the more endocrine-disruptive components, and an understanding of this process will be an important component in the formulation of environmental risk assessments for this group of products.

Bacteria have been credited with three broad strategies for primary biodegradation of nonionic (polyethoxylate) surfactants (36): separation at the ether bond joining hydrophilic to hydrophobic moieties (central fission); ω/β-oxidative degradation of the hydrophobic group; and successive exocision of the hydrophilic group. In mixed cultures, the first and second strategies fail for NPEO$_x$ due to, respectively, the proximity of the aryl nucleus and the presence of branching in the nonyl chain, leaving only the last mechanism (36). This is consistent with the observed accumulation of residues with intact nonylphenol moieties in natural environments.

Laboratory studies with mixed cultures (20, 23, 31) and pure cultures (15, 24, 26) have demonstrated that biodegradation results in the conversion of NPEO to shorter homologs, usually nonylphenol diethoxylate (NPEO$_2$) and/or its carboxylic acid analog, and it has been tacitly assumed that this is achieved by sequential removal of C$_2$ ethoxylate units from the end of the chain. However, definitive evidence for the operation of this pathway in pure culture is lacking, and the nature of the ether scission reaction which it requires is unknown. The
work now described was undertaken to isolate bacteria capable of growth on NPEO surfactants, to confirm that growth was at the expense of only the ethoxylate moiety, and to elucidate the pathway and mechanism for ethoxylate removal.

MATERIALS AND METHODS

Materials and reagents. NPEO, surfactant mixtures Ethyl 77, Ethyl BCN, and Ethyl 20, kindly donated by Ackcross Chemicals Ltd., Manchester, United Kingdom, contained branched-chain nonylphenols attached to polydisperse polyethylene glycol chains averaging 6, 9, and 20 glycol units and were designated NPEO, NPEO, and NPEO, respectively. Pure dodecanol ethoxylates CxEOy, CxEOy, and CxEOy were obtained from Nikkol Chemical Company, Tokyo, Japan. All other reagents, including high-pressure liquid chromatography (HPLC) grade hexane, isopropanol, methanol, and acetonitrile, were from Fisher Scientific UK, Loughborough, United Kingdom.

Isolation, maintenance, and growth of bacteria. Activated sewage sludge from Dyffryn Sewage Treatment Works, South Glamorgan, United Kingdom, was used to inoculate sterile basal salts medium (pH 7) containing 8.1% (wt/vol) NPEO as the sole added carbon source. Basal salts contained (grams/liter) K2HPO4 (3.5), KH2PO4 (1.5), NaCl (0.5), MgCl2·6H2O (0.15), and Na2SO4·H2O (0.34) plus trace elements (1 ml/liter) containing (grams/liter) NaHCO3 (10.0), MnSO4·4H2O (0.3), ZnCl2·(CHO3)·2H2O (0.2), NH4NO3·4H2O (0.02), CuSO4·5H2O (0.5), Al2(SO4)3·6H2O (0.15), CoCl2·6H2O (0.15), and Fe(NH4)2(SO4)2·6H2O (0.5). Cultures were agitated at 30°C and 150 rpm.

Cultures were transferred to fresh medium, individual colonies were isolated on plates containing the same medium solidified with Noble agar. Axenic strains were maintained on agar slants of the same medium.

Bacterial isolates were shown to be gram negative (29) and further identified by using BIOLOG GN multwell plates (BIOLOG Inc., Hayward, Calif.). Bacteria were grown routinely in liquid basal salts medium supplemented with surfactants as required. Growth was measured by removing 1-ml samples from culture flasks, adding methanol (50% [vol/vol], final concentration) to dissolve insoluble hydrophobic products of biodegradation, and then measuring optical density at 600 nm. Separate experiments demonstrated that cells were unaffected by the presence of this concentration of methanol.

Preparation and use of cell lysate. Pseudomonas putida was grown in 2-liter flasks containing 500-ml batches of 0.1% (wt/vol) NPEO and basal salts medium (pH 7) containing 8.1% (wt/vol) NPEO as the sole added carbon source. Basal salts contained (grams/liter) K2HPO4 (3.5), KH2PO4 (1.5), NaCl (0.5), MgCl2·6H2O (0.15), and Na2SO4·H2O (0.34) plus trace elements (1 ml/liter) containing (grams/liter) NaHCO3 (10.0), MnSO4·4H2O (0.3), ZnCl2·(CHO3)·2H2O (0.2), NH4NO3·4H2O (0.02), CuSO4·5H2O (0.5), Al2(SO4)3·6H2O (0.15), CoCl2·6H2O (0.15), and Fe(NH4)2(SO4)2·6H2O (0.5). Cultures were agitated at 30°C and 150 rpm. After several transfers to fresh medium, individual colonies were isolated on plates containing the same medium solidified with Noble agar. Axenic strains were maintained on agar slants of the same medium.

Acetic acid used was of distilled water and used for media preparation. 0.02 ml of ultrapure water. Ten milliliters of the stock reagent was added to 1 ml of orthophosphoric acid. 10 ml of 20% (vol/vol) barium chloride to make the spray reagent. While not fully quantitative, this TLC method was invaluable for rapid assessment of biodegradability and for evaluating metabolic patterns arising from commercial mixtures and pure surfactants.

HPLC measurement of parent surfactants and metabolites. HPLC for analysis of NPEO homologs was performed essentially by the method of Ahel and Giger (1), using a normal-phase Lichrosorb 10-μm irregular packed NH2-bonded silica column (4.6 mm [inside diameter] by 250 mm; Phenomenex, Macclesfield, United Kingdom). The automated HPLC system comprised Milton-Roy Consta Metric III metering pumps, an LDC/Milton-Roy Spectromonitor D detector set at 254 nm, a 50-μl injection loop connected to a UV injector device and accessory control module controlled by a Commodore CBM model 8050 processor with an MP3000 integrator. Elution was achieved at the flow rate of 1.5 ml/min with solvents A (n-hexane-isopropyl alcohol, 9:1 [vol/vol]) and B (isopropanol/ultrapure water, 9:1 [vol/vol]) in linear gradients between the following limits: at t = 0, A = 93% and B = 7%; at t = 60 min, A = 37% and B = 63%; at t = 62 min, A = 93% and B = 7%; at t = 67 min, A = 93% and B = 7%. Surfactant samples from biodegradation experiments were prepared for injection by centrifugal microfiltration in 0.75-ml, 0.2-μm pore-size microfiltration centrifuge tubes (Lida Manufacturing Corp., Kenosha, Wis.) and centrifuged for 5 min at 3,000 rpm on a bench microcentrifuge.

Ahel and Giger (1) showed that the molar extinction coefficients were identical for the nonylphenol homologs, including nonylphenol itself (x). Therefore, from the molar extinction coefficient of nonylphenol and the HPLC peak areas derived from standard solutions, the molar amounts of each NPEO homolog were calculated.

HPLC of glycolaldehyde and acetaldehyde. The method of Benassi et al. (9) for the detection of formaldehyde as its 2,4-dinitrophenylhydrazine derivative, was modified for use with acetaldehyde and glycolaldehyde. To derivatize the aldehydes, 1-ml aliquots of standard or test samples were added to 9 ml of tetrahydrofuran and 0.06 ml of 2,4-dinitrophenylhydrazine (0.1% [wt/vol] in 1 M HCl), vortex mixed for 20 s, and allowed to settle for 2 min on ice before the addition of 0.8 ml of 0.1 M phosphate buffer (pH 7.4) and 1.4 ml of 1 M NaOH. Derivatized samples were stored on ice prior to HPLC analysis. Derivatized glycolaldehyde and acetaldehyde were separated by a Lichrosorb RP18 column (4.6 mm [inside diameter] by 250 mm; PhaseSep, Desdeide, United Kingdom) installed in a Dionex 300 ion chromatograph fitted with a Dionex 4400 integrator (Dionex UK, Camberley, United Kingdom) and an LDC Milton Roy Spectromonitor IV detector (9554 nm). Samples were injected isocratically in acetonitrile-water (2:3 [vol/vol]) at a flow rate of 1 ml/min.

Assay for acetaldehyde. Acetaldehyde in solution was determined by reaction with 3-methyl-2-benzothiazoline hydrazone in the presence of ferric chloride according to the method of Barary et al. (8).

GC-MS of biodegradation end products. A sample (1 ml) of spent culture fluid from the biodegradation of NPEO by P. putida was acidified to pH 2 in 1 M HCl (HPLC grade; Fisher) and extracted into chloroform (HPLC grade) by vortex mixing for 20 s. The lower chloroform layer was removed, dried over anhydrous sodium sulfate (HPLC grade), and analyzed by gas chromatography-mass spectrometry (GC-MS) using a Hewlett-Packard 5890 HP Series II gas chromatograph fitted with an Automated Hewlett-Packard Injector 7673 and a silicon HP-5 column (30 m by 0.25 mm) and interfaced to a 100-sample automated 5971A mass spectrometer. The conditions were as follows: 1-μl injection volume, carrier gas, helium (0.5 ml/min); temperature gradient, 50 to 170°C at 30°C/min, 170 to 240°C at 5°C/min, and 240 to 280°C at 2°C/min, isothermal for 8 min; total run time, 46 min. The ionization energy and ionizer temperature for electron impact ionization were 70 eV and 285°C, respectively.

RESULTS AND DISCUSSION

Isolation of bacteria. Enrichment cultures yielded two strains of the genus Pseudomonas, each of which was confirmed for its ability to utilize, as sole source of carbon and energy for growth in liquid culture, NPEO and NPEO but not NPEO.
polyoxyethylene glycol or nonylphenol. In view of their close similarity in these respects, results are presented here only for one strain, which was further identified as *P. putida* type 1A1 (degrees of similarity with BIOLOG database were 0.910 and 0.935 for 4- and 24-h readings of color development in BIOLOG GN plates).

**Utilization of NPEO*<x*> as growth substrate.** Figure 2 shows the growth curves and surfactant disappearance as measured by CTAS for *P. putida* growing separately on each of the three surfactants NPEO<sub>av6</sub>, NPEO<sub>av9</sub>, and NPEO<sub>av20</sub>. For the shortest homolog (Fig. 2c), the disappearance of surfactant (CTAS) was simultaneous with growth. In contrast, for the longest homolog tested (NPEO<sub>av20</sub> Fig. 2a), extensive growth occurred before there was any depletion of surfactant. The CTAS assay responds well to, but is unable to discriminate among, the longer homologs, and it is progressively less sensitive to shorter homologs, the total peak area remained constant throughout.

Available carbon for growth from each surfactant mixture (NPEO<sub>av6</sub>, NPEO<sub>av9</sub>, and NPEO<sub>av20</sub>) was calculated from HPLC analysis of each mixture, first, on the basis that degradation proceeded to NPEO<sub>1</sub>. On this basis, the growth yields in stationary phase were correlated with the available carbon with <i>r</i><sup>2</sup> = 0.9998. When available carbon was recalculated on the basis that degradation proceeded to NPEO<sub>2</sub>, NPEO<sub>3</sub>, NPEO<sub>4</sub>, or NPEO<sub>5</sub>, the value of <i>r</i><sup>2</sup> was 0.9998, 0.9977, 0.9916, or 0.9814, respectively. The highest (and indistinguishable) values of <i>r</i><sup>2</sup> for NPEO<sub>4</sub> and NPEO<sub>5</sub> provided evidence that degradation proceeded at least to the diethoxylate.

**GC-MS analysis of end product of biodegradation of NPEO<sub>av9</sub>.** To determine the structure of the end product of the biodegradation of NPEO<sub>av9</sub>, this surfactant was degraded to completion by *P. putida*, and the end product was analyzed by GC-MS. The gas chromatogram showed three peaks (a to c) eluting close together (retention times of 21.55 [a], 21.94 [b], and 22.30 [c] min). In spectrum a, the molecular ion was 308, corresponding to NPEO<sub>5</sub>. Confirmation of this identity was provided by the fragmentation pattern which centered largely around fragmentation of the nonyl chain. Thus, the major ions with <i>m/z</i> values of 279, 237, 223, and 209 correspond to ([M + 1] – n(CH<sub>2</sub>OH), where <i>n</i> = 2, 5, 6, and 7. Spectrum b contained the same ions and additionally <i>m/z</i> = 251, corresponding to <i>n</i> = 4. Spectra a and b were closely similar to those obtained by Maki et al. (24) for (1,3,3-trimethyl-1-ethyl butyl)phenol diethoxylate and (1,1,4,4-tetramethyl pentyl)phenol diethoxylate, respectively. Spectrum c (Fig. 3) shares these same major ions and also contains the <i>m/z</i> = 265 ion corresponding to loss of a three-carbon fragment from NPEO<sub>2</sub>; we suggest this is a third isomer, (1,4-dimethyl-1-ethyl pentyl)phenol diethoxylate (Fig. 3). Whatever the isomeric structures of the nonyl group, the major conclusion from these data is that the diethoxylate NPEO<sub>2</sub> accumulates as the major end product of NPEO<sub>av9</sub> biodegradation in *P. putida*.

**Biodegradation kinetics of component homologs of NPEO<sub>x</sub>.** The identification by GC-MS of NPEO<sub>2</sub> as the accumulating end product of biodegradation of NPEO<sub>x</sub> enabled the corresponding HPLC peak to be identified. It is reasonable to assume that successively eluting peaks in the HPLC chromogram differed by single ethylene glycol units, thereby enabling assignment of all the higher homologs in the series.

Availability of a fully standardized HPLC system enabled quantitative determination of the biodegradation kinetics of each component homolog in a commercial mixture. The observed pattern for biodegradation of NPEO<sub>x</sub> by *P. putida* (Fig. 4) showed the longest homologs (NPEO<sub>13</sub> and NPEO<sub>14</sub>) disappearing progressively from the outset, while intermediate homologs were more persistent and NPEO<sub>2</sub> accumulated as the major end product. The accumulation of NPEO<sub>2</sub> could occur by either (i) cleavage of all homologs at the ether bond from longer homologs would result in no change in CTAS response until the chains had been shortened significantly. Therefore, these data provided preliminary evidence that *P. putida* degraded NPEO<sub>x</sub> via successive exoscission of the polyethoxylate chain rather than by central cleavage.

The final cell yields (Fig. 2) increased in the order NPEO<sub>av6</sub> < NPEO<sub>av9</sub> < NPEO<sub>av20</sub>, and there was no growth on nonylphenol itself. These results strongly implied that the carbon for growth was derived exclusively from the ethoxylate chain, a conclusion confirmed (i) by measurements of the UV absorbance of cell-free culture media which remained constant throughout growth and (ii) by HPLC analysis (with UV detection) of intermediates formed during biodegradation, which showed that as the ethoxamers were degraded to shorter homologs, the total area remained constant throughout.
between the second and third glycols from the phenyl ring (EO\textsubscript{2–3} cleavage), (ii) sequential removal of single glycol units so that each homolog is eroded to the NPEO\textsubscript{x} structure (exoscission), or (iii) scission at ether links within the polyethylene glycol chain (endoscission). For exclusive EO\textsubscript{2–3} cleavage, all homologs would decrease simultaneously, albeit possibly at different rates, but there would be no increases in amounts of any but the end product itself. In contrast, for sequential exo-cleavage of single glycol units or endoscission, the longest homolog could decrease only in amount, but intermediate-length homologs would be undergoing formation from longer ones and degradation to shorter ones and thus may achieve a steady state for a while, or even increase in amounts if the rate of formation exceeded the rate of biodegradation. This condition would persist until the homolog became the longest remaining, and then it would decrease in amount. It is clear from Fig. 4 that intermediate homologs frequently showed small increases, for example, at 8 and 10 h for NPEO\textsubscript{11}, at 10 h for NPEO\textsubscript{19} at 8 h for NPEO\textsubscript{9}, NPEO\textsubscript{3}, and NPEO\textsubscript{7}, at 6 h for NPEO\textsubscript{9}, and at 10 and 12 h for NPEO\textsubscript{4}, NPEO\textsubscript{2}, and NPEO\textsubscript{3}. Similar results were obtained with NPEO\textsubscript{av6} and NPEO\textsubscript{av20}. This pattern eliminated the EO\textsubscript{2–3} scission mechanism, leaving endo- or exoscission of the polyethoxylate chains as remaining possibilities. Because the organism did not utilize free polyethylene glycols, endoscission of NPEO\textsubscript{x} would have resulted in the accumulation of polyethylene glycols. No such accumulation was ever detected on TLC plates with the Burger reagent (12), which indicates that the mechanism of polyethoxylate degradation was by exoscission.

**Activity of NPEO\textsubscript{x} degraders toward AEO\textsubscript{x}**. The difficulty in interpreting the data in Fig. 4 is that the immediate product of exo-glycol cleavage of a particular component was already present in the mixture and itself undergoing biodegradation. Although the pure NPEO\textsubscript{x} components needed to resolve this problem were unavailable, pure AEO\textsubscript{x} components were available and were used as follows. Cells of *P. putida* grown on NPEO\textsubscript{av9} harvested and reincubated with the growth substrate, achieved complete biodegradation as assessed by TLC analysis. However, broth-grown cells were completely without activity. This finding indicated that the ether scission system was inducible by substrate or a transient metabolite. Moreover, NPEO\textsubscript{av9} grown cells, but not broth-grown cells, degraded the pure alcohol ethoxylate AEO\textsubscript{8}, which indicated that the ether scission system induced by and active on NPEO\textsubscript{av9} was also active on AEO\textsubscript{8}. This allowed the use of the pure compounds
AEO₈, AEO₇, and AEO₅ in mechanistic studies, thus avoiding the complications from homolog mixtures.

Extracts of NPEOav₉-grown cells were incubated with AEO₈, and samples were removed at intervals and analyzed by TLC using the Burger reagent to reveal polyethylene glycol-containing compounds (Fig. 5). Samples of AEO₈, AEO₇, and AEO₅ were also chromatographed as standards. The first sample was removed immediately after mixing cells with substrate; during the time taken to process this sample, a small amount of conversion occurred to give a single new spot corresponding to AEO₇. After 30 min of incubation, AEO₇, AEO₆, and small amounts of AEO₅ were present, but shorter homologs did not appear until the 90-min sample. The facts that no short homologs were formed initially and that AEO₇, AEO₆, and AEO₅ showed clear precursor-product relationships confirmed that degradation proceeded by stepwise exosclission of single glycol units and not by endosclission. The same results were obtained under aerobic and anaerobic conditions.

Acetaldehyde production from NPEOav₉ in cell extracts. The two most likely mechanisms for liberation of glycol units from NPEO are (38) (i) hydroxyl (HO) shift from the terminal −CH₂OH to the penultimate carbon to yield the hemiacetal −O−CH(OH)CH₃ followed by spontaneous decomposition of the hemiacetal to acetaldehyde and (ii) insertion of oxygen via monoxygenation to produce the hemiacetal −O−CH(OH)−CH₂OH, which on decomposition yields glycolaldehyde. Thus, identification of the aldehyde product by HPLC is a suitable discriminator for these mechanisms. With 2,4-dinitrophenylhydrazine used to derivatize the aldehydes and HPLC to separate and identify the resulting UV-absorbing hydrazones, no aldehydes were detectable in the culture medium containing whole cells biodegrading NPEO₉. This was not surprising because it is normal for cells which generate toxic aldehydes to keep the steady-state concentration very low by having efficient systems for aldehyde removal (7). In contrast, cell extracts in which onward metabolism was disrupted were capable of significant biodegradation of NPEO₉ (monitored by TLC analysis), and moreover they produced abundant acetaldehyde but neither glycolaldehyde nor any other aldehyde (by HPLC analysis of 2,4-dinitrophenylhydrazones). The same results were obtained under aerobic conditions and after purging all solutions with nitrogen.

To confirm that oxygen was not required for acetaldehyde production, cell extracts were sparged with nitrogen and then incubated under a nitrogen atmosphere with nitrogen-sparged AEO₈ solution (final concentration of approximately 2 mM). Samples were removed at intervals and assayed for acetaldehyde by the method of Barary et al. (8). The results showed that the acetaldehyde concentration reached 3.5 mM after 2 h. This is much higher than the oxygen concentration present even in air-saturated water and therefore much higher still than in the nitrogen-sparged solution actually used. Thus, the acetaldehyde must be liberated in a mechanism which is independent of oxygen.

Mechanism of biotransformation. The combined evidence presented in this paper, viz. (i) analysis of metabolite patterns from NPEO₉ mixtures, (ii) precursor-product relationships for biodegradation of the pure ethoxamer of AEO₈ via AEO₇, AEO₆, and AEO₅, and (iii) liberation of acetaldehyde, shows...
toward polyethylene glycol itself. The previously reported systems because it lacks any activity over the biodegradation of nonionic polyethoxylate surfactants. Moreover, the dehydration of the terminal glycol unit and hydrolysis (without liberation of acetaldehyde). This mechanism is dependent on ether scission (Fig. 6) accounts for the oxygen-independent mechanism in NPEO. The proposed nonoxidative hydroxyl shift mechanism of ether scission (Fig. 6) accounts for the oxygen-independent liberation of acetaldehyde. This mechanism is dependent on the availability, in the polyethoxylate, of a terminal hydroxyl group which is effectively transferred to the penultimate carbon to produce the labile hemiacetal. The anaerobic Pelobacter venetianus and Bacteroides strain PG1 (16) degrade polyethylene glycol to acetaldehyde by a similar hydroxyl shift mechanism. To account for a similar oxygen-independent degradation of polyethylene glycol to acetaldehyde by Acinetobacter, Pearce and Heydeman (28) proposed a mechanism involving dehydration of the terminal glycol unit and hydrolysis (without hemiacetal formation) to yield the enol form of acetaldehyde and the next-lower polyethylene glycol homolog. Although this mechanism is possible, the data are also consistent with the hydroxyl shift mechanism.

While the hydroxyl shift mechanism shown in Fig. 6 clearly has precedents in bacterial metabolism of polyethylene glycols, this is the first report of the occurrence of such a mechanism in the biodegradation of nonionic polyethoxylate surfactants. Moreover, the P. putida enzyme system must be quite distinct from the previously reported systems because it lacks any activity toward polyethylene glycol itself.

The sequential exocission of glycol units from NPEO is consistent with the study of Kvetstak and Ahel (20), who showed the formation of shortened but unoxidized NPEO homologs by mixed estuarine cultures. On the other hand, some workers (4, 24) have interpreted the frequent occurrence of oxidized intermediates (carboxylic acid anhydras) in mixed culture and environmental samples as indicative of the importance of oxidizing mechanisms in NPEO biodegradation. A parallel situation exists in the biodegradation of polyethylene glycols, for which there is evidence for their oxidation to carboxylic acids but no evidence on which to assess whether oxidation is a precursor to, or a component of, the ether scission step (38). It may be that nonoxidative ether scission dominates NPEO (and polyethylene glycol) biodegradation to shorter homologs and that the longer-lived intermediates undergo hydroxyl group oxidation as a side reaction, possibly mediated by alcohol dehydrogenases known to occur ubiquitously in bacteria.

In environmental samples, although NPEO accumulates significantly, its carboxylic acid analog and the more endocrine-disrupting nonylphenol are also found (3, 4, 11, 14, 17, 21, 22, 25). These transformations were not observed in P. putida, and so other organisms or possibly abiotic processes must be involved to achieve these conversions in the environment.

The progressive shortening of ethoxylate chains during biodegradation will make the molecules more lipophilic, which is expected to increase adsorption of these surfactants to organic-rich sediments and to decrease affinity for more polar mineral components in natural sediments. Adsorption may in turn influence the bioavailability of the biodegradation intermediates to further biodegradation by bacteria. Thus, the successive exocission mechanism established in this study is likely to have significant implications in assessing the environmental fate of this group of surfactants.

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