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Aerobic Transformation of Short-Chain Alkylphenol Polyethoxylates by Mixed Bacterial Cultures

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Abstract. Various aerobic mixed bacterial cultures, isolated from wastewater, river water, and from a forest soil, were applied for the biotransformation of short-chain alkylphenol polyethoxylates (APnEO; nEO = 1–3) using the shake culture technique. Almost complete transformation of both nonylphenol- and octylphenol polyethoxylate mixtures, added to a synthetic sewage at total concentrations in the range of 0.5–2.5 mg/L, was achieved within 6–23 days. The duration of the adaptation period (lag phase) and the transformation rate in exponential phase varied significantly, depending on the origin of the bacterial culture and structural characteristics of the APnEO molecules (number of EO groups per molecule and the size of the alkyl chain). Experiments conducted in a mineral medium showed that short-chain APnEO can be transformed even if they represented the only source of organic carbon, though at a rate significantly lower than that in the synthetic sewage. Autochthonous bacterial cultures originating from a polluted river and a secondary sewage effluent were also able to transform short-chain APnEO, the transformation rate being strongly dependent on temperature. The analyses of shake culture at the end of the exponential phase revealed a significant presence of alkylphenoxy carboxylic acids which accounted for up to 90% of APnEO originally added to the growth-media. This suggested that carboxylation of the terminal alcohol group was the most important transformation process involved.

The biodegradation of nonionic surfactants is determined by two key elements of their chemical structure: (a) the number of EO groups in the hydrophilic chain and (b) the structure of the hydrophobic group (Swisher 1987). The differences in biodegradation kinetics and metabolic pathways observed for the two main groups of nonionic surfactants, alcohol polyethoxylates (LAEO) and alkylphenol polyethoxylates (APnEO; Figure 1), indicate the predominant impact of the hydrophobic moiety on their biodegradation behavior. It is commonly accepted that LAEO represent easily and ultimately degradable compounds both in sewage treatment (Kravetz *et al.* 1982) and natural waters (Larson and Games 1981). In contrast, "assessment of the biodegradability of the alkylphenol ethoxylates has given rise to more disagreement, contradiction and controversy than any other area of surfactant degradation" (Swisher 1987). A great deal of the disagreement and controversy can be explained by an uncritical application of non-specific analytical methods for APnEO determination or by insufficient adaptation of the applied bacterial cultures.

Although complex metabolic pathways of APnEO have not been completely understood, the majority of authors agree that their biotransformation starts on the hydrophilic part of the molecule by cleaving-off C_2-unit at a time (Swisher 1987). The major mechanisms involved in the biotransformation of the EO-chain are hydrolytic ether cleavage and oxidation of the terminal OH group (Swisher 1987; Ball *et al.* 1989). Application of modern analytical techniques, such as high-resolution gas chromatography and high-performance liquid chromatography, resulted in the past decade in the discovery of rather persistent toxic metabolites that are formed from APnEO (Giger *et al.* 1981; Reinhard *et al.* 1982; Stephanou and Giger 1982; Brüschweiler *et al.* 1983; Giger *et al.* 1984; Ahel *et al.* 1987). Therefore, increasing attention is presently being paid to reevaluation of the environmental acceptability of APnEO. A special emphasis is put on assessing the environmental behavior and fate of their metabolic products.

Biotransformation of the higher APnEO oligomers (nEO = 3–20) in sewage treatment was shown to be relatively fast and efficient (Giger *et al.* 1987; Ahel 1987) resulting, however, in the formation of various stable metabolites which represented more than 60% of the total alkylphenolic compounds that entered sewage treatment. Approximately 30% of the total nonylphenolic compounds determined in secondary effluents were found in the form of lipophilic metabolites, including short-chain APnEO (nEO = 1–2) and fully de-ethoxylated products (alkylphenols). Consequently, their concentrations in freshwaters that receive inputs from secondary effluents can be significant (Ahel 1987; Ahel *et al.* 1991). Due to their comparatively enhanced persistence towards biotransformation, increased toxicity to the aquatic organisms and high consumption

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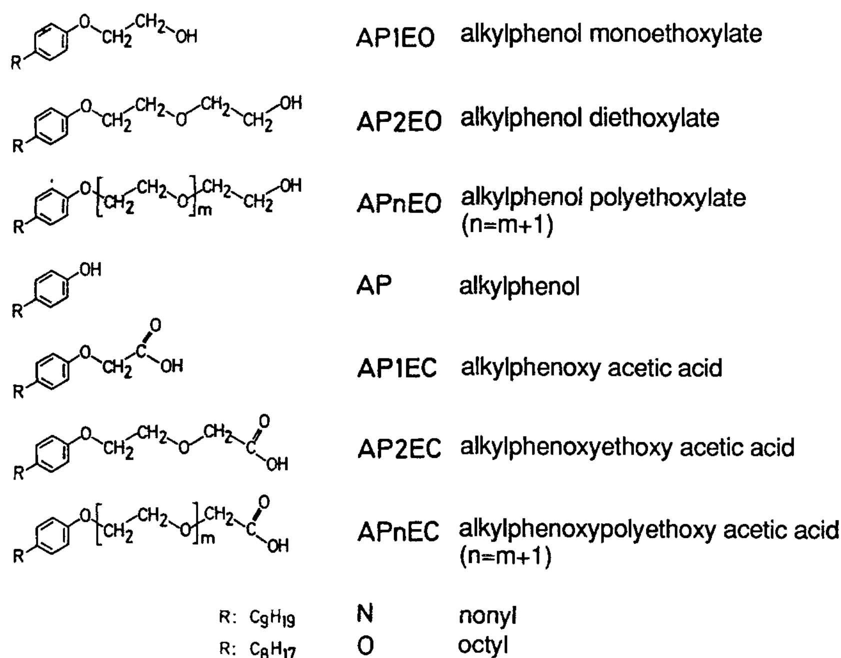

Fig. 1. Structures and acronyms of alkylphenol polyethoxylates and their metabolites

rate of their parent compounds, short-chain APnEO are pollutants of high environmental concern. However, very few studies were specifically dedicated to investigate the biodegradation behavior of short-chain APnEO (Holt *et al.* 1991). In a comprehensive study by Ball *et al.* (1989) halogenated and non-halogenated octylphenol polyethoxylates (nEO = 1–5) were subjected to biotransformation under aerobic and anaerobic conditions. The results indicated formation of various alkylphenolic transformation products under both conditions, while the time needed for the complete biotransformation exceeded 100 days. In our study a comparison is given between the biotransformation behavior of the short-chain octyl- and nonylphenol polyethoxylates under aerobic conditions using mixed bacterial cultures of different origin.

Materials and Methods

Chemicals

The following commercial mixtures of alkylphenol polyethoxylates were used in biotransformation experiments:

—Imbetin N/7A (Dr. W. Kolb AG, Hedingen, Switzerland): a mixture of nonylphenol polyethoxylates containing 75% of NP1EO, 20% of NP2EO and 5% of NP3EO; the nonyl-chain is highly branched.
—4-*Tert*-octylphenol-2Aeo (Dr. W. Kolb AG, Hedingen, Switzerland): mixture of octylphenol polyethoxylates containing 27% of OP1EO, 46% of OP2EO, 18% OP3EO and 9% OP4EO.

The APnEO solutions were prepared by equilibrating 1 g of the commercial mixtures with 5 L of distilled water with slow stirring. The formed concentrated solutions, were used to prepare appropriate dilutions for biotransformation experiments.

Media and Bacterial Cultures

Two types of growth media were applied in the shake culture test: synthetic sewage and a mineral medium after Horvath and Koft (1972). The synthetic sewage contained the following constituents: NaCl (7 mg/L), $CaCl_2{:}2H_2O$ (4 mg/L), $MgSO_4{:}7H_2O$ (2 mg/L), K_2HPO_4 (28 mg/L), pepton (160 mg/L), and meat extract (110 mg/L).

The mineral growth medium was prepared by dissolving the following components in 1 L distilled water: NH_4Cl (1 g), $Na_2HPO_4{:}12H_2O$ (1 g), KCl (0.5 g) and $MgCl_2{:}6H_2O$ (0.18 g). Mercury(II) chloride (1% solution in distilled water) was used as an antibacterial agent for the control experiment.

Bacterial cultures originated from three different sources: wastewater of a detergent manufacturing plant (Saponia, Osijek, Croatia), chronically polluted river water (Drava River, near Osijek), and a pristine forest soil. A detailed description of the enrichment technique used to isolate these bacterial cultures has been described elsewhere (Karuza-Stojaković 1984). Briefly, bacterial cultures were grown in a synthetic sewage medium which contained 20 mg/L of a commercial NPnEO mixture (Hoechst, Frankfurt, Germany; average EO number of 9) by using continuous-flow units of 300 ml (dilution rate of 0.1 h^{-1}). During five weeks of cultivation by successively increasing NPnEO concentration up to 325 mg/L, the bacterial cultures originating from river water and wastewater revealed high degradation efficiency at the highest concentration level while the culture from the forest soil was less efficient. The enriched cultures were stored at −16°C in a solution containing 40% of glycerol, 0.33 g/L of meat extract, and 7.5 mg/L of the NPnEO commercial mixture. Before being used in the biodegradation experiments the stored cultures were grown in the synthetic sewage containing 5 mg/L of NPnEO over a period of 2 weeks as described above. One-week-old cultures were used as inoculum in the shake culture test.

Shake Culture Test

Sterile 500-ml Erlenmeyer flasks containing 200 ml of previously autoclaved synthetic sewage or mineral medium were spiked with a known concentration of APnEO compounds (typically 0.5–1.0 mg/L of NPnEO and 2.0–2.5 mg/L of OPnEO) and inoculated with one of the selected mixed bacterial cultures. After the addition of inoculum the cultures which served as sterile controls were poisoned by adding 1 ml of 1% mercury(II) chloride solution. For each experiment two parallel series of identical samples were prepared.

The flasks were placed on a shaker situated in a thermostated room at

23.5°C. At the defined time intervals, sample aliquots for the high-performance liquid chromatographic (HPLC) determination of APnEO (10 ml) and for bacteriological analysis (determination of the number and type of bacteria) were taken from each flask under sterile conditions. The number of the bacteria was determined by the standard method of counting developed colonies (colony forming units = CFU) on the surface of Nutrient Agar (Difco, MI, USA). At the end of the exponential phase, when the major part of added APnEO was transformed, the total content of a duplicate sample (200 ml) was analyzed for biotransformation products. After termination of the experiment, the flask walls were rinsed with *n*-hexane in order to determine the amount of adsorbed APnEO.

The biotransformation rate constants (k_b) of individual APnEO in the exponential phase was calculated assuming first-order kinetics according to expression:

$$k_b = -\ln(C/C_0)/t$$

where C_0 and C stand for APnEO concentrations at the beginning and moment t, respectively.

River Water Test

Die-away tests were conducted using autochthonous bacterial cultures from a polluted river and secondary effluent. The experiments were performed in two modifications: (a) static method (no stirring) and (b) with stirring (modified river water test). In the first experiment, river water collected from the Sava River near Zagreb (BOD = 3 mg/L; dissolved oxygen = 8 mg/L) was artificially polluted with Imbetin N/7A at the concentration of 1.1 mg/L. The die-away test was performed in dark bottles of 1.3 L at the room temperature [20(±)2°C]. In certain time intervals, 10-ml aliquots were taken for the HPLC determination.

In the second die-away experiment APnEO biotransformation was examined using secondary sewage effluent from sewage treatment plant Dübendorf, Switzerland, containing 90 μg/L NP1EO and 64 μg/L NP2EO. A 40 L sample of the effluent was divided into three 20-L glass vessels and kept under two different temperatures: 4°C and 20°C. The subsample poisoned with formaldehyde solution (10 mL/L) served as a control. Prior to HPLC analysis 1-L aliquots of these media were extracted using a steam distillation-solvent extraction apparatus (Veith and Kiwus 1977).

Analytical Methods

The details on the analytical protocols used for the determination of lipophilic APnEO and their carboxylated metabolic products by high-performance liquid chromatography (HPLC) and high-resolution gas chromatography/mass spectrometry (HRGC/MS) have been reported elsewhere (Ahel and Giger 1985; Ahel 1987; Ahel *et al.* 1987). Briefly, shake culture aliquots (10 ml) were extracted in graduated glass vials applying 1 min shaking with *n*-hexane at a solvent to sample ratio 1:10. The phase separation was attained by a 2 min centrifugation of 2000–3000 rpm and the known amount of internal standard (2,4,6-trimethylphenol, 99%, EGA-Chemie, Steinheim, Germany) was added into the *n*-hexane phase. The concentrations of individual APnEO oligomers were determined by normal-phase HPLC.

Authentic standards were used to quantify individual components. Individual oligomers of APnEO were obtained by preparative HPLC from their commercial mixtures, while APEC were prepared by oxidation of APnEO mixtures, followed by isolation of individual APEC by preparative normal-phase HPLC (Ahel and Giger 1985; Ahel 1987; Ahel *et al.* 1987).

The liquid chromatograph consisted of a Waters high-pressure pump (Model 45) equipped with a Rheodyne syringe loading sample injector (Model 7125) and a Kratos Spectroflow 773 spectrophotometric detector. The chromatographic column applied (Dr. Knauer, Berlin, Germany) was a 100 mm × 4 mm i.d. column packed with spherical particles of aminosilica (Hypersil® APS, 3 μm). All HPLC determinations were performed in the isocratic mode using a mixture of *n*-hexane and 2-propanol as a mobile phase (2 ml/min). Detection wavelength was set at 277 nm.

The samples for HRGC/MS analyses (200 ml) were extracted with chloroform at a pH value of 2 in order to enable the recovery of acidic metabolites. The extracts were dried with sodium sulphate, evaporated to dryness followed by the methylation of the residue by 1 N HCl in methanol. A Carlo Erba gas chromatograph (Model 4160) connected to a Finnigan mass spectrometer (Model 4021 C) equipped with a INCOS 2000 data processing system was used. The setting of the mass spectrometer was as follows: ionization energy 70 eV; ion source temperture 270°C; analyzer pressure 1.1×10^{-6} torr; sensitivity 10^{-7} A/V; mass range 45–480 daltons; scanning time 1 s. After a splitless injection glass capillary gas chromatographic column (SE-52, 22 m) was programmed from 50°C to 270°C at a rate of 4°C/min.

Results

Shake Culture Test in the Synthetic Sewage Medium

The biotransformation of NP1EO and NP2EO (added in the form of commercial mixture Imbetin N/7A) in the synthetic sewage using the shake culture test is shown in Figure 2. Biotransformation in the exponential phase of both oligomers was relatively fast, depending on the culture used as inoculum. The bacterial cultures from the river water and wastewater showed similar biotransformation rates ($k_b = 0.23–0.33\ d^{-1}$), while the culture isolated from soil proved somewhat less efficient ($k_b = 0.18–0.21\ d^{-1}$). In the experiment with the initial number of bacteria of 10^6 CFU/mL (Figure 2a,b) the lag phase was not obvious (less than 1 d), while in an identical experiment, but with a smaller initial number of the bacteria (10^4 CFU/ml), biotransformation started after a lag phase of 1 d (Figure 2c,d). In this phase the number of bacteria increased to 10^6 CFU/ml. Consequently, the transformation rates in the exponential phase for these two experiments were rather similar. It is also interesting to note that there were no conspicuous differences between biotransformation rates of NP1EO and NP2EO.

Abiotic elimination of approximately 10% observed in the control experiment (Figure 2a,b) was not considered significant. Up to 10% of the total APnEO added to the medium was adsorbed on the Erlenmeyer flask walls. The percentage was constant during the experiment only for the sterile controls whilst in the active bacterial cultures no detectable concentrations of the adsorbed APnEO were found at the end of the experiment. This indicated that a dynamic equilibrium existed between APnEO adsorbed on the glass walls and APnEO in the solution.

Figure 3 shows the biotransformation of the octylphenol ethoxylates (nEO = 1–3) in synthetic sewage using also the shake culture technique. The biotransformation rate gradually decreases from OP3EO ($k_b = 0.24–0.64\ d^{-1}$) towards OP1EO ($k_b = 0.09–0.21\ d^{-1}$). This is especially evident in the experiment with the bacterial culture isolated from forest soil which exhibits comparatively lower ability to transform APnEO and

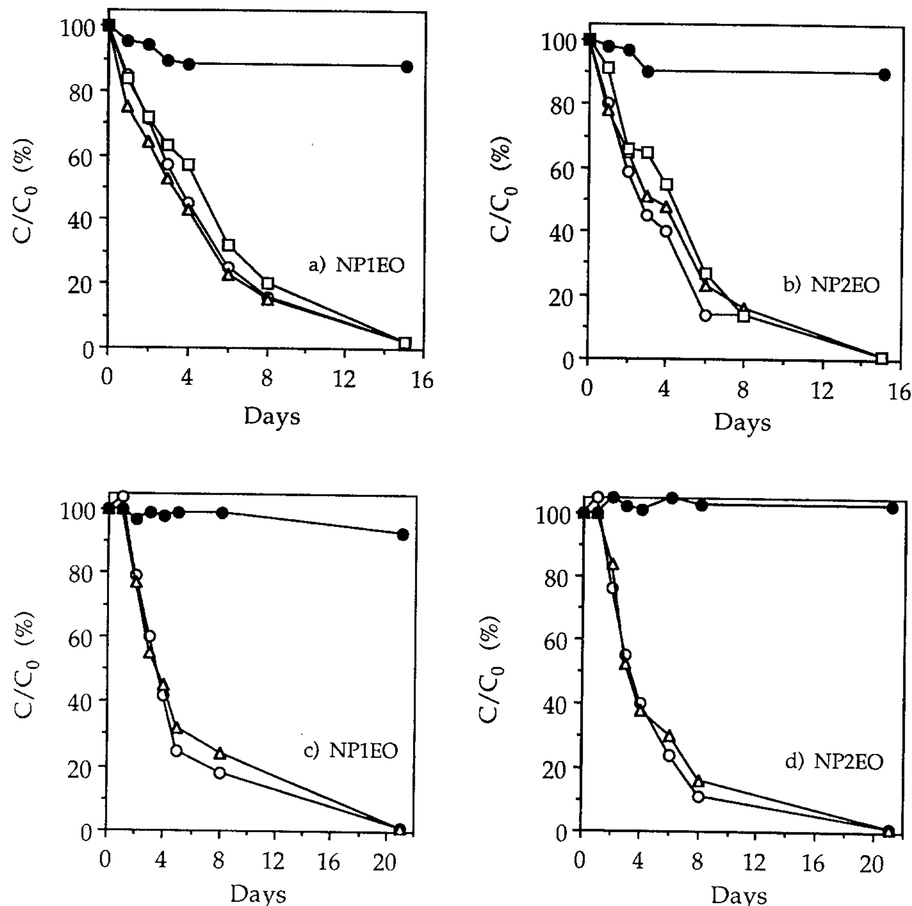

Fig. 2. Biotransformation of short-chain nonylphenol polyethoxylates in synthetic sewage using the shake culture test. Inoculum: (○) bacterial culture from river water, (△) bacterial culture from the wastewater of a detergent manufacturing plant, (□) bacterial culture from a forest soil, and (●) control culture (50 mg/L $HgCl_2$ added). Initial number of bacteria: (a and b) 10^6 CFU/ml; (c and d) 10^4 CFU/ml. Initial concentration (C_0): (a and b) NP1EO = 0.34 mg/L and NP2EO = 0.10 mg/L; (c and d) NP1EO = 0.40 mg/L and NP2EO = 0.12 mg/L

consequently slower transformation kinetics. Actually, between the second and the fifth day, a complete stagnation in the OP1EO biotransformation was observed in the flask containing the culture from forest soil. It can be inferred that this bacterial culture utilized firstly easier degradable OP3EO and OP2EO, and only then OP1EO. In addition, it cannot be exluded that some OP1EO was formed metabolically from higher oligomers.

Comparison of the biotransformation rates for different APnEO homologues *i.e.*, OPnEO and NPnEO, is not possible in a strict sense since their concentrations as well as the composition of oligomers in the applied commercial mixtures were not identical. Nevertheless, it seems that there was no large difference between the biotransformation rates of the octyl and nonyl homologues when the same bacterial cultures were used.

Shake Culture Test in the Mineral Medium

Experiments performed in a completely mineral medium were aimed to examine whether APnEO can be transformed if they represented the sole source of organic carbon. Applied bacterial cultures were identical to those from the experiments with the synthetic sewage. The corresponding biotransformation curves of NP1EO and NP2EO are shown in Figure 4. Apparently, the biotransformation rate in the mineral medium was much slower than that in the synthetic sewage (Figure 2) which is very probably a consequence of the lower biomass ($<10^4$ CFU/ml) in the former. However, comparison of the transformation rates obtained for different bacterial cultures confirmed similarities between the cultures isolated from wastewater and river water (k_b = 0.16–0.20 d^{-1}) as well as relative inferiority of the culture isolated from forest soil (k_b = 0.06–0.12 d^{-1}).

General conclusions which can be drawn from the experiment with OPnEO (Figure 5) are identical to those discussed above for NPnEO, but the picture is more complex due to the higher number of oligomers involved. The difference between transformation rates of individual oligomers for different bacterial cultures is less pronounced.

The impact of the alkyl-chain size on the biotransformation rates was rather small as indicated by a comparison of analogous OPnEO and NPnEO oligomers.

River Water Test

In order to provide a better assessment of APnEO behavior in natural waters, die-away tests were carried out using autochthonous bacterial cultures originating from a polluted river and from a secondary sewage effluent. The results presented in Figure 6a show biotransformation of NP1EO and NP2EO added to the river water in concentrations of 800 μg/L and 300 μg/L, respectively. As can be seen, relatively efficient biotransformation of NP1EO and NP2EO was obtained in the static test (open symbols). The remaining concentrations decreased exponentially with no evident lag phase. Biotransformation rates in the experiment using stirring (k_b = 0.35–0.37 d^{-1}) were 1.5 times faster than in the static one (k_b = 0.23

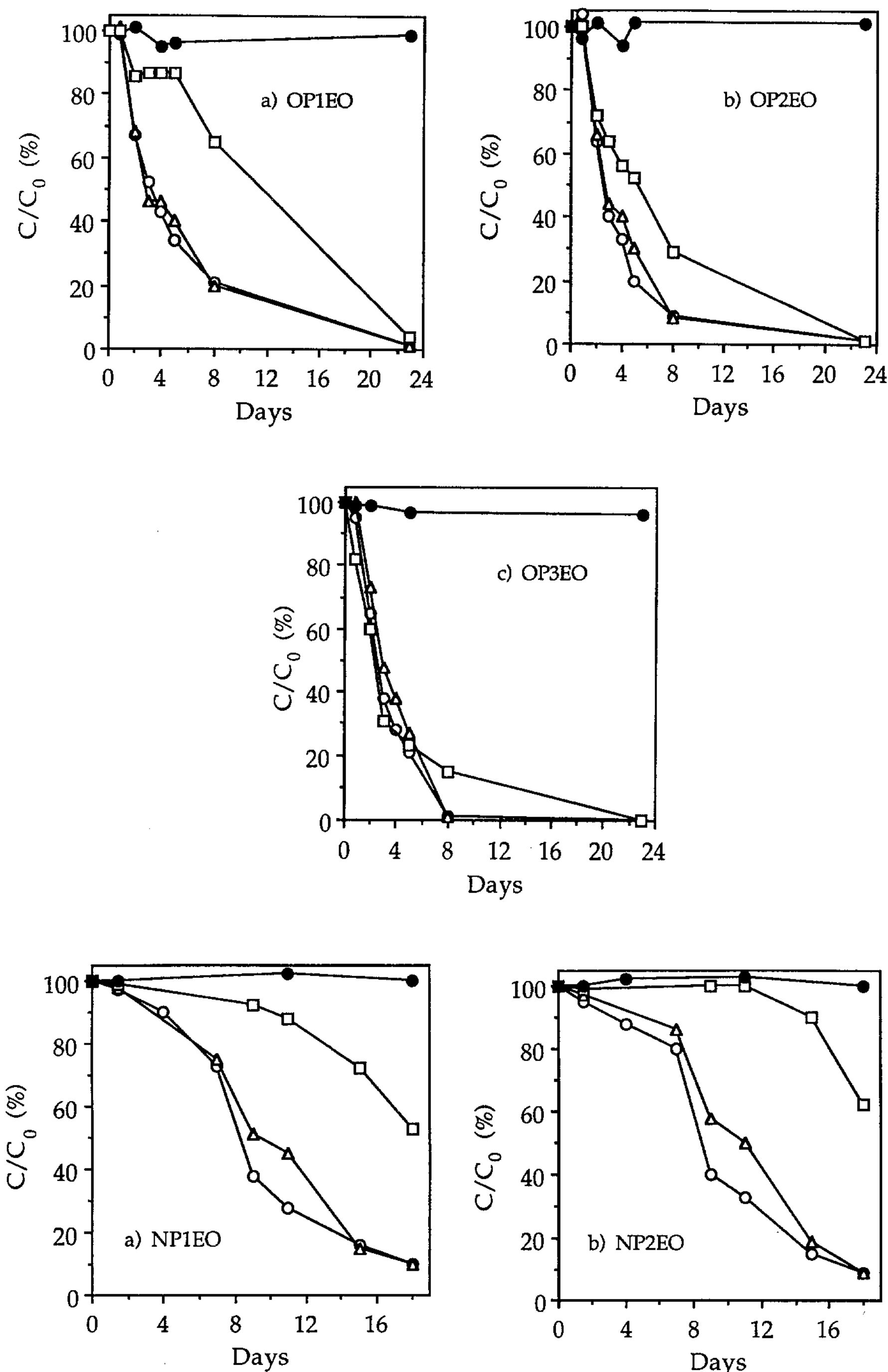

Fig. 3. Biotransformation of short-chain octylphenol polyethoxylates in synthetic sewage using the shake culture test. Inoculum: (○) bacterial culture from river water, (△) bacterial culture from the wastewater of a detergent manufacturing plant, (□) bacterial culture from a forest soil, and (●) control culture (50 mg/L $HgCl_2$ added). Initial number of bacteria: 10^4 CFU/ml. Initial concentration (C_0): OP1EO = 0.59 mg/L, OP2EO = 0.87 mg/L, and OP3EO = 0.45 mg/L

Fig. 4. Biotransformation of short-chain nonylphenol polyethoxylates in mineral medium using shake culture test. Inoculum: (○) bacterial culture from river water, (△) bacterial culture from the wastewater from a detergent manufacturing plant, (□) bacterial culture from a forest soil, and (●) control culture (50 mg/L $HgCl_2$ added). Initial number of bacteria: 10^4 CFU/ml. Initial concentration (C_0): NP1EO = 0.75 mg/L and NP2EO = 0.25 mg/L

d^{-1}). This could probably be explained by an enhanced transfer of oxygen in the solution.

Figure 6b shows die-away kinetics of NP1EO and NP2EO in a secondary sewage effluent sample (initial concentrations of 90 μg/L and 64 μg/L, respectively) kept at two different temperatures (4 and 20°C). In subsamples containing active bacterial populations NP1EO and NP2EO were transformed at very similar rates. As expected, biotransformation at the higher temperature (k_b = 0.09 d^{-1}) was more efficient than at the lower one (k_b = 0.01 d^{-1}). After 12 d, only 10–20% of NP1EO and NP2EO were transformed at 4°C, compared to 50–60% at 20°C. A control subsample to which 1% of formaldehyde was added showed no change in the analyte concentrations during the whole experiment (not shown in Figure 6).

Biotransformation Products of APnEO

Towards the end of the exponential phase of the shake culture (8–10 d after the experiment started) culture media were analysed for biotransformation products using HRGC/MS. In all of the samples examined alkylphenoxy carboxylic acids (APnEC)

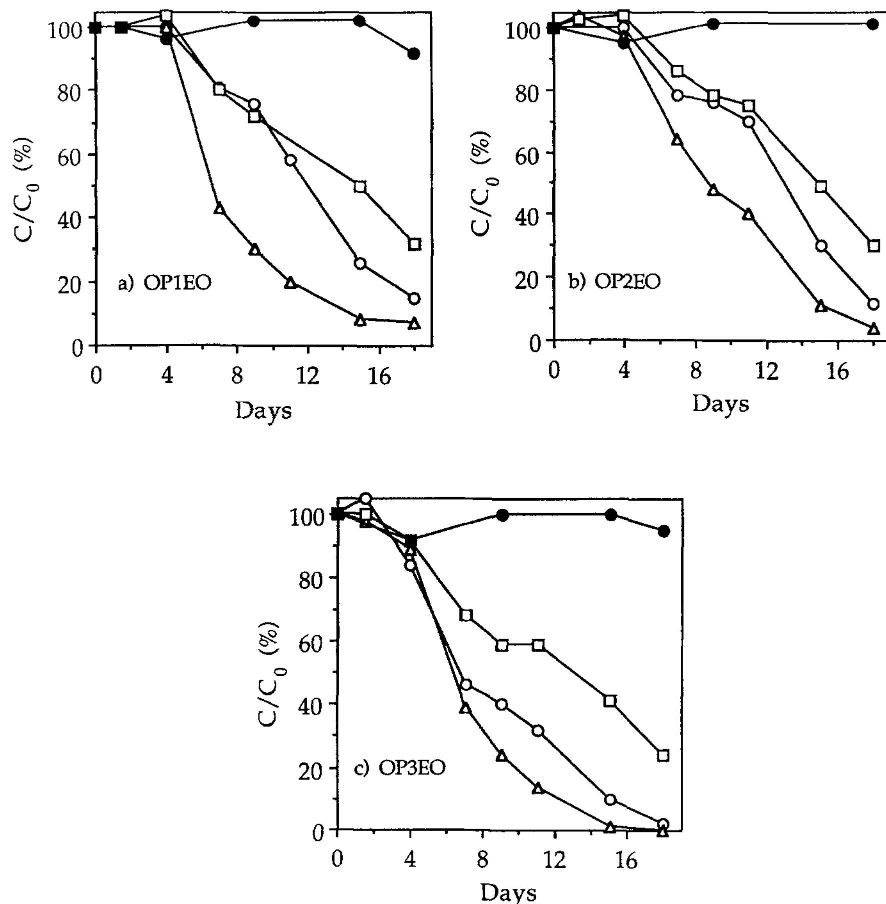

Fig. 5. Biotransformation of short-chain octylphenol polyethoxylates in mineral medium using the shake culture test. Inoculum: (○) bacterial culture from river water, (△) bacterial culture from the wastewater from a detergent manufacturing plant, (□) bacterial culture from a forest soil, and (●) control culture (50 mg/L $HgCl_2$ added). Initial number of bacteria: 10^4 CFU/ml. Initial concentration (C_0): OP1EO = 0.63 mg/L, OP2EO: 1.03 mg/L, and OP3EO: 0.51 mg/L

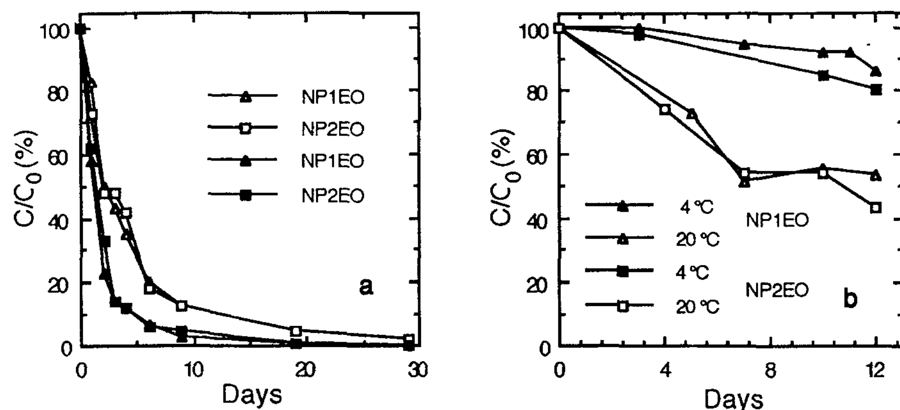

Fig. 6. Biotransformation of nonylphenol mono- (NP1EO), and diethoxylate (NP2EO) using the river water test. (a) Autochthonous culture from river water. Initial concentration (C_0): NP1EO = 800 μg/L; NP2EO = 300 μg/L; open symbols: static test, filled symbols: with stirring. (b) Autochthonous culture from a secondary sewage effluent. Initial concentration (C_0: NP1EO = 90 μg/L and NP2EO = 64 μg/L. Stirring was applied at both temperatures

were found to be the dominant constituents of the extracts. The only compounds which appeared at similar concentrations to APnEC were common fatty acids. Figure 7 shows total ion current chromatograms of typical samples containing biotransformed NPnEO (A) and OPnEO (B) mixtures. The mass spectra of the identified NPnEC and OPnEC are discussed in detail elsewhere (Ahel *et al.* 1987; Reinhard *et al.* 1982). In the experiment with NPnEO, NP1EC and NP2EC were identified as biotransformation products corresponding to NP1EO and NP2EO, which were originally present in the medium. Similarly, in the experiment with commercial mixture of OPnEO, structural identifications were made for OP1EC, OP2EC, OP3EC, and OP4EC, reflecting the oligomer composition of the test mixture. Moreover, the respective relative abundances of individual NPnEC and OPnEC were very similar to those of the nontransformed APnEO mixtures. Unfortunately, APnEC were not quantitatively determined simultaneously with APnEO during the whole biotransformation experiment. Nevertheless, the results obtained at the end of the exponential phase (Table 1) showed considerable concentrations of APnEC which accounted for more than 90% of the APnEO originally added to the media. The extracts were thoroughly examined for other possible transformation products, but not even traces of such metabolites were identified.

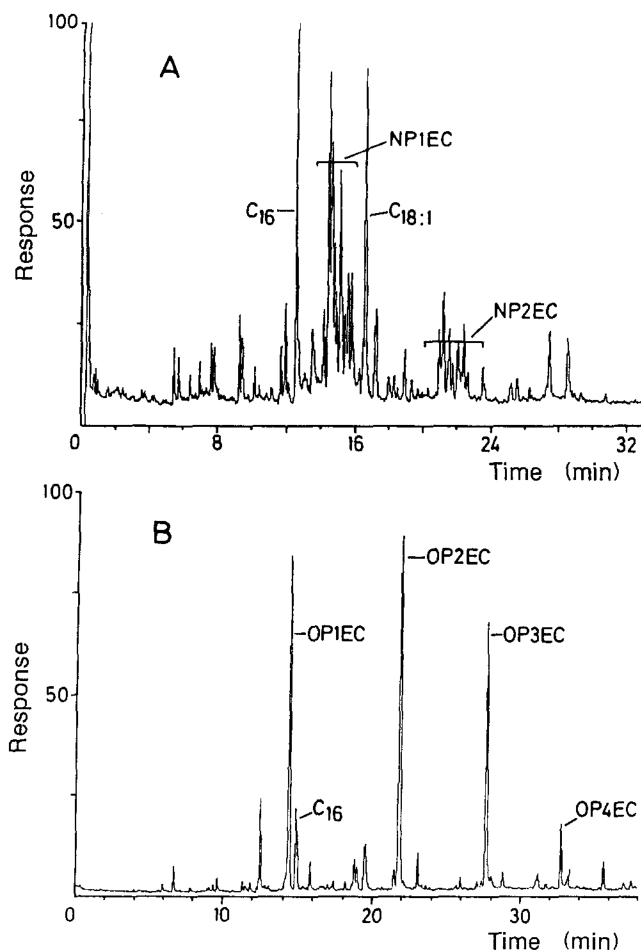

Fig. 7. Total ion current chromatograms of the total chloroform extracts obtained from the shake cultures, which originally contained NPnEO and OPnEO, 8 days after beginning the biotransformation experiments presented in Figure 2c,d (A) and Figure 3 (B). NP1EC-NP2EC: nonylphenoxy carboxylic acids. OP1EC-OP4EC: octylphenoxy carboxylic acids. C_{16}, $C_{18:1}$: common fatty acids. The extract was methylated before HRGC/MS analysis

Discussion

Short-chain APnEO (nEO = 1–3), which are usually considered as persistent lipophilic metabolites from the aromatic nonionic surfactants, were efficiently transformed under aerobic conditions using various mixed bacterial cultures isolated from different habitats (Figures 2 and 3). Similar biotransformation rates were observed using the bacterial cultures isolated from river water and wastewater of a detergent manufacturing plant, while significantly lower efficiency was obtained for the bacterial culture isolated from a pristine forest soil. This observation can be explained by the fact that the first two locations are known to be chronically polluted with APnEO which allowed the autochthonous bacterial population to adapt to this type of xenobiotic compounds. The fact that even the bacteria from a pristine nonaqueous environment were able to biotransform APnEO indicates that there is quite a number of bacterial species which can be adapted for such transformation. In addition, transformation was shown to occur even in the mineral medium where APnEO were the only source of organic carbon (Figures 4 and 5). Therefore, these compounds cannot be regarded as trully persistent compounds under aerobic conditions. Even an autochthonous bacterial population from chronically polluted river was efficient in transforming NP1EO and NP2EO at rather high concentrations (Figure 6a). Surprisingly, the transformation of APnEO at a lower concentration using bacterial population from a secondary sewage effluent was much slower (Figure 6b). Since it is reasonable to assume that this bacterial population was well adapted to APnEO, this indicates that some other factors, such as copresence of other substrates or inhibiting substances, can have a strong impact on the biotransformation process. It is also important to note that APnEO transformation was strongly dependent upon temperature, suggesting that their degradation in the aquatic environment during winter could significantly be slowed down. Indeed, a significant reduction of the "elimination" rate of NP1EO and NP2EO were observed in the winter period both in sewage treatment (Ahel 1987) and natural waters (Ahel *et al.* 1991).

Table 1. Composition of alkylphenol polyethoxylates (APnEO) and alkylphenoxy carboxylic acids (APnEC) in synthetic sewage at the beginning of the experiment and at the end of exponential phase of the shake culture test. Inoculum 1 and 2: mixed bacterial cultures isolated from river water and forest soil, respectively

Compound	Initial concentration (μg/L)	Inoculum 1 Final conc. (μg/L)	Inoculum 1 (%)[a]	Inoculum 2 Final conc. (μg/L)	Inoculum 2 (%)[a]
NP1EO	400	20[b]	5	40[b]	10
NP2EO	120	6[b]	5	10[b]	8
NP1EC	<1	360[b]	86	260[b]	62
NP2EC	<1	67[b]	53	17[b]	13
OP1EO	590	30[c]	5	<1[d]	0
OP2EO	870	18[c]	2	<1[d]	0
OP3EO	450	<1[c]	0	<1[d]	0
OP1EC	<1	560[c]	90	580[d]	93
OP2EC	<1	850[c]	93	640[d]	70
OP3EC	<1	450[c]	96	380[d]	81

[a]Expressed as percentage of the initial concentration of corresponding APnEO

[b,c,d]Culture media were analyzed 10, 8, and 23 days, respectively, after beginning the shake culture test

The enhanced resistance of the short-chain APnEO towards microbial transformation as compared with their higher oligomers is well documented for both real-scale and laboratory systems (Rudling and Solyom 1974; Geiser 1980; Brüschweiler *et al.* 1983; Ahel 1987). Biodegradation of APnEO mixtures having an average EO number of 10 and containing only traces of the short-chain oligomers (nEO < 3) was shown to result in a complete depletion of the higher oligomers accompanied by an accumulation of AP1EO and AP2EO. The increased persistence of these short-chain oligomers is usually interpreted as the consequence of their enhanced lipophilicity caused by shortening the hydrophilic moiety. This generally applicable, but very simplified, concept fails to explain why AP2EO were the strongly predominant ethoxylated biotransformation product from both OPnEO and NPnEO. For example, the octanol/water partition coefficients for NP2EO and NP3EO are almost identical (Ahel and Giger 1993), but the concentration of NP2EO in biologically treated effluents is several times higher than that of NP3EO (Ahel 1987). Furthermore, if the existence of a critical

lipophilicity threshold was the reason for the inhibition of further transformation, a significant difference should be seen between the metabolic patterns of OPnEO and NPnEO oligomer mixtures, since the lipophilicity in terms of octanol/water partition coefficients is approximately three times higher for NPnEO as for OPnEO (Ahel and Giger 1993). Consequently, the parameter determining the persistence of the short-chain APnEO can be described more precisely as the distance of the terminal alcohol group from the branched alkylbenzene moiety, which is closely related with the Griffin's hydrophile-lipophile balance (HLB) value (Griffin 1954). The experiments shown in the present work are in agreement with such a hypothesis since no significant difference was observed between the biodegradation rates of corresponding NPnEO and OPnEO oligomers.

The biodegradation rate in the OPnEO series decreased gradually from OP3EO to OP1EO. It should be noted that each of the higher APnEO oligomers is a possible precursor for the formation of the lower ones. Therefore, the apparent transformation rates of individual oligomers represent actually differences between their transformation and formation rates. Consequently, a small difference in the biotransformation behavior of the examined OPnEO and NPnEO mixtures can possibly be explained by their slightly different oligomer compositions. Commercial mixture Imbetin N/7A contained virtually only NP1EO and NP2EO, representing 75% and 20%, respectively, of the total. In contrast, the commercial mixture OPnEO contained oligomers in the range of 1–4 EO with OP2EO as the predominant constituent (46% of the total).

A prolonged lag phase of the OP1EO transformation, which was observed with bacterial culture isolated from soil (Figure 3), suggested that bacteria start first to transform easier degradable higher oligomers. This effect was not as obvious with other isolates because the transformation rate of all oligomers was much faster which minimized the possibility to observe differences for individual compounds. Similar observations regarding the preferential biotransformation of the highest OPnEO present in the medium were made by Ball *et al.* (1989) who used activated sludge and primary sewage as inoculum.

Mechanisms which determine such a behavior are as yet not fully understood. According to biotransformation products identified in our experiment (Figure 8), it seems that the oxidation of the terminal alcohol group was the principal mechanism of the initial biotransformation for both OPnEO and NPnEO. The concentration of APnEC at the end of the exponential phase corresponded to a major part of APnEO added in the media while their relative abundance closely resembled the original composition of individual APnEO oligomers. This suggests that the alternative transformation mechanism involving hydrolytic ether cleavage of either APnEO or APnEC was of minor importance. The extracts were carefully examined by GC/MS for other possible transformation products to check the hypothesis of APnEO biotransformation via oxidation of the alkyl chain as postulated by Schöberl *et al.* (1981). No such metabolites were found which would contain carboxylated moiety in the alkyl side chain. A study by Kravetz *et al.* (1982) using tritium-labelled NPnEO suggested a substantial transformation of the aromatic ring, but no corresponding intermediates has been identified so far. Our experiments are consistent with some earlier observations (Ahel *et al.* 1987; Ball *et al.* 1989) on the high persistence of carboxylated intermediates of the short-chain APnEO under aerobic conditions.

The typical value for the biotransformation half-life of short-chain APnEO in our biotransformation experiments was 2–3 d. Values reported in the literature range from few hours to weeks (Ball *et al.* 1989). Obviously, aerobic mixed bacterial cultures selected for our experiments cannot be regarded as representative for whole range of environmental conditions. However, our results indicate that short-chain APnEO can readily be biotransformed in aerobic environments such as secondary sewage treatment and natural waters chronically polluted with surfactants. Field studies on NPnEO behavior in sewage treatment and natural waters (Ahel 1987) support this conclusion. The half-life of NP1EO and NP2EO of approximately 1–2 days, estimated from our study in the Glatt River, Switzerland (Ahel *et al.* 1991), is in very good agreement with the values obtained from the laboratory experiments presented in this work. Moreover, a mass balance of all nonylphenolic compounds suggest that most of the NPnEO were transformed into nonylphenoxy carboxylic acids, which exhibited a rather persistent behavior in the river (Ahel 1987).

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