Anaerobic degradation of nonylphenol mono and diethoxylates in municipal solid waste and landfilled sludge

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INTRODUCTION

Nonylphenol ethoxylates (NPEOs) are extensively used as surfactants in industry products. Degradation products of alkylphenol ethoxylates are potentially bioaccumulated and, thereby becoming toxic to aquatic organisms (Lewis 1991), and soil microorganisms (Trocmé et al. 1988). Partial degradation of NPEOs can proceed both aerobically and anaerobically, and although the metabolic pathways are not completely understood, it is believed that biotransformation commence at the hydrophilic part of the molecule and that C-2 units are removed one at a time (Swisher 1987). Complete degradation of these short-chain NPEOs appears to be possible under aerobic conditions (Ekelund et al. 1993; Ahel et al. 1994a), while they have been reported as more persistent in anaerobic environments (Giger et al. 1984; Marcomini et al. 1989). In the present study we investigated the anaerobic biotransformation and degradation of nonylphenol mono and diethoxylates (NPEO1 and NPEO2) in landfilled municipal solid waste samples and landfilled sludge samples. This investigation was initiated by Akzo Nobel Surface Chemistry AB and the Swedish Environmental Protection Agency.

MATERIALS AND METHODS

To investigate the potential for microbial transformation of NPEO2 two inocula were used. Each inoculum was exposed to 2, 60 and 308 mg l^{-1} of mixtures of NPEO1 and NPEO2. Experimental bottles incubated with the mixture of NPEO1 and NPEO2 were frozen periodically to assess possible transformation of the added compounds. The first inoculum originated from a municipal solid waste landfill (Filborna Landfill, Helsingborg, Sweden). The second inoculum was taken from a landfill used for disposal of dewatered sludge from an anaerobic sludge reactor treating waste waters containing NPEOs. The preparation and inoculation procedures was done in triplicate according to Ejlertsson et al. (1996). The total solid content (TS) in the experimental bottles after addition of MSW- or sludge inoculum was 2 g l^{-1} and 5 g l^{-1}, respectively. NPEOs dissolved in methanol was added to experimental bottles directly after inoculation to give concentrations of 2, 60 and 308 mg l^{-1}. To account for abiotic degradation two sets of triplicate

bottles were sterilised by autoclavation and incubated. All incubations were made in triplicate and placed in the dark at 30 °C.
Gas samples (0.3 ml) for methane analysis were withdrawn from the experimental bottles and quantified by gaschromatography as described by Örlygsson et al. (1993). Analysis of bottles with NPEOs at 60 and 308 mg l^{-1} was done essentially according to Ejlertsson et al. (1997). Botles at 2 mg l^{-1} was done essentially according to Wahlberg et al. (1990).

RESULTS AND DISCUSSION

The distribution among the NP, NPEO1 and NPEO2 in the NPEO mixture studied were determined with GC/MS. The distribution was found to be about 0,15 % NP, 70 % NPEO1, 28 % NPEO2 and a few per cent NPEO3.
Microorganisms from both the landfilled reactor sludge and the municipal solid waste landfill had the potential for NPEO degradation at 2 mg l^{-1} (Fig. 1). The degradation of the added NPEOs resulted in an accumulation of NP, which most likely was not further mineralised. MSW-inoculum degraded the NPEOs added in 24 days, whereas it took about 50 days in the landfilled reactor sludge inoculum to perform degradation of NPEOs. NP was also formed from NPEOs added at higher concentrations 60 mg l^{-1} (Table 2). In bottles inoculated with MSW at 60 mg l^{-1}, the transformation of NPEOs appeared to be concentrated to the first 30 days of incubation. No transformation of NPEOs was observed with 308 mg l^{-1}. Bottles with the landfilled sludge degraded NPEOs at 60 and 308 mg l^{-1}, during concomitant formation of NP. However, analysis for NP and NPEOs were made only at day 0 and 154 for this inoculum.

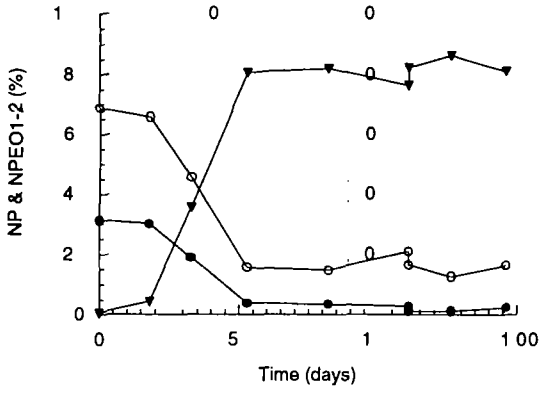

Figure 1. Formation of NP (P) during transformation of NPEO1 (E) and NPEO2 (J) at 2 mg l^{-1} in bottles inoculated with landfilled sludge. The Y-axis shows the relation between NP, NPEO1 and NPEO2 in the extracts analysed

Our observations of the degradation of NPEOs in this study are in line with earlier reports (Giger et al. 1984; Marcomini et al. 1989) showing that under anaerobic conditions NPEOs can be degraded to NP, which is far more persistent than their parental compounds.
The methanol added was completely degraded by both inocula studied and contributed with 187 µmoles of the methane produced in experiment bottles. In the labelled experimental bottles the contribution of methane was 660 µmoles. In the landfilled sludge inoculum, CH_4 production in the NPEO amended samples were at the levels of the controls. The amount of CH_4 formed ranged between 350-420 µmol. With the MSW-inoculum, the pattern of CH_4 production was different (Fig. 2). Samples amended with 2 mg l^{-1} of NPEOs followed the controls closely. In experimental bottles with 308 mg l^{-1} NPEOs, the CH_4 production was equal to the controls for the first couple of weeks. After that CH_4 production became inhibited. After a period of ca 80 days the CH_4 production resumed and at the termination of the experiment the amounts were

equal to those found in the controls (approximately 300 µmol CH_4). Samples amended with 60 mg l^{-1} NPEOs were also inhibited with regard to CH_4 production, but to a lesser extent. We do not have an explanation for this response, but the NPEOs might adversely effect the acetate utilising methanogens, and thereby partially hamper the methane formation.

Table 2. Formation of NP in experiment bottles inoculated with landfilled MSW- and sludge samples at the different concentrations of NPEOs. The degradation is expressed as per cent of each homologue of the total amount of NP, NPEO1 and NPEO2 at every sampling occasion. bd=below detection limit.

Inocula (Time days)	NP (%)	NPEO1 (%)	NPEO2 (%)
Municipal solid waste			
60 mg l^{-1}			
0	bd	70	30
23	39	51	10
92	41	43	17
122	36	47	17
154	17	76	7
Landfilled sludge			
60 mg l^{-1}			
0	bd	71	29
154	68	32	

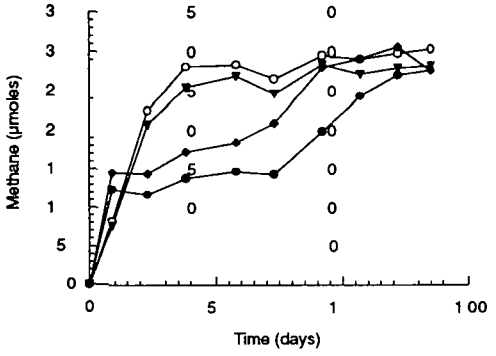

Figure 2 Formation of methane in bottles inoculated with MSW-samples. Symbols represent methane formed in control bottle (E), 2 mg NPEOs l^{-1} (P), 60 mg NPEOs l^{-1} (F) and 308 mg NPEOs l^{-1} (J).

Addition of NPEOs did not result in an increase in the total amount of CH_4 formed in any of the samples, further indicating that the phenol ring structure remained intact during the whole period of incubation. It is conceivable, however, that the EO-groups liberated by degradation of higher NPEOs could be further degraded to CH_4. Glycol, the product formed during NPEO hydrolysis, may be degraded to methane and carbon dioxide. The possible amount of glycol to be released

would, however, be to low to generate enough methane to contrast over the levels produced from indigenous substrates in the inocula.
The experimental design chosen for this study displays a few built in characteristics which, unfortunately, hinders the evaluation of the results to some degree: The fact that NPEO1 and NPEO2 are strongly hydrophobic limits the biological availability of the compounds. The heterogeneity concerning the chemical and physical properties of the inocula used, may also give rise to their limited availability for microbial attack due to adsorption. Furthermore, the compounds showed a tendency to become attached to the glass walls of the bottles used for the experiment. Also, the inoculates typically contains an array of complex organic and inorganic properties of unknown structure, which may disturb the analysis. However, the fact that NP was formed at the expense of NPEO1 and NPEO2 shows a microbial potential for NPEO degradation in the inocula used. From studies with ^{14}C labelling of the rings of the NPEOs (to be published elsewhere), it is also clear that NP was not further degraded since ^{14}C marked NPEOs were recovered at almost 100% at the termination of the experiment. This was supported by the lack of any ^{14}C marked CH_4 or CO_2 in the headspace of bottles with ^{14}C labelled NPEOs.

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