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Fate and Behaviour of Alkylphenols and their Poly-ethoxylates in an Activated Sludge Plant

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Alkylphenol polyethoxylates (APEOs) are commercially important non-ionic surfactants used in industrial and domestic detergent and emulsifier formulations (Ferugerson et al. 2001), and nonylphenol ethoxylates (NPEO) and octylphenol ethoxylates (OPEO) are some of the most commonly used surfactants. The exposure of wildlife to these chemicals is mainly through water by discharge through wastewater treatment plant effluents and possibly sewage disposal (Montgomery-Brown and Reinhard 2003; Ying et al. 2002). It has been reported that partial biodegradation of NPEO in wastewater treatment plants generates more persistent nonylphenol (NP) and shorter-chain mono- to triethoxylates (NP₁₋₃EO) (Giger et al. 1984). NPEOs and their degradation products have been detected in effluents of many municipal sewage treatment works (STWs) and their concentrations demonstrate spatial variation from below detection limits to 343 µg l⁻¹ (Ying et al. 2002). Laboratory studies have also demonstrated the build up of NP in biological sludge due to its recalcitrance and hydrophobic nature (Langford et al. *unpublished data*). The wide-spread occurrence of NP₁₋₃EO in the environment (Ferguson et al. 2001; Elke F and Wilhelm P 2004) is of concern, as studies have shown that NPEO metabolites (NP and NP₁₋₃EO) are more toxic and endocrine disrupting than their parent substance, through mimicking natural hormones by interacting with the estrogen receptor (Jobling et al. 1996; Renner 1997). As a consequence of these observations, scientific and regulatory concerns have been raised over the occurrence of (NP and NP₁₋₃EO) in the environment above the threshold necessary to disrupt endocrine function in wildlife.

Considering the growing emphasis on the quality of water as exemplified by the Water Framework Directive (2000/60/EC) and water reuse issues worldwide (Gomes 2003) this study reports on the transformation of APEO within the activated sludge process (ASP) and the removal efficiency of the process for NP and NP₁₋₄EO in a United Kingdom STW. As the biological suspended solids in the activated sludge may represent a sink for these more hydrophobic metabolites a mass balance was undertaken to allow for elucidation of factors involved in controlling discharge of NP and the shorter chain ethoxymers.

MATERIALS AND METHODS

Both the settled sewage and final effluent were sampled at 6 hour intervals while return or waste activated sludge (RAS/WAS) was sampled once a day. Filtration of suspended solids and solid phase extraction (SPE) were performed at a facility adjacent to the activated sludge treatment plant, which has been described (Jones et al. *unpublished data*).

Suspended solids in each sample were determined according to standard methods (HMSO 1980). To facilitate extraction of the dissolved NPEO by SPE and to collect the solids for quantification of NPEO, samples were filtered through GFC A (VWR, Lutterworth, UK), then were stored in resealable plastic bags and frozen. The tC18 SPE cartridges (Waters, Watford, UK) were preconditioned with methanol before use. Sample volumes depended on sample type: settled sewage (100 ml); final effluent (250 ml); the RAS/WAS (100 ml) was centrifuged at 1000g to facilitate collection of bulk solids then filtered prior to SPE. On return to the laboratory, dissolved phase samples, pre-sorbed onto SPE cartridges in the field, were eluted and solids were defrosted and air-dried prior to extraction and quantification by LC/MS.

Pure polyethoxylate compounds were not commercially available, therefore commercial mixtures (Aldrich, Poole, UK) were used to prepare standards for calibration. A mixture of Igepal CO520 and CO720 were used to prepare a mixture of NPEOs for quantification, with Igepal CA210 and CA720 used for the OPEOs (Langford et al. 2004). Nonylphenol was quantified as 4-nonylphenol and octylphenol as 4-(tert-octyl)-phenol. Stock solutions of 1000 mg l⁻¹ were prepared in acetonitrile (ACN) and working standards were prepared by dilution with 50/50 ACN/water. Quantification by LC/MS used negative mode for NP, monitored as [M-H]⁻, and NPEOs were determined in positive mode as sodium adducts [M+Na]⁺.

RESULTS AND DISCUSSION

The concentrations of OPEO entering the secondary treatment process averaged 7.2 µg l⁻¹ over the 4 day sampling period, compared to the 151.5 µg l⁻¹ observed for the NPEO. Therefore, the octyl ethoxylates comprised ~ 5% of total inputs, compared to up to 20% observed by others (Staples et al. 1999). As the concentrations of OP and the OPEO were significantly below those of the nonyl

compounds, the results and discussion focuses on data obtained for the latter group, however, this would be expected to apply equally to OPEOs as degradation pathways are the same (Ahel et al. 1994) and where appropriate data relating to the OPEO is referred to in support of this.

The concentrations of NP₉₋₁₀EO in the settled sewage were those of other oligomers in the aqueous phase with an average of 25.6, 24.8 and 21.3 $\mu\text{g l}^{-1}$ respectively over the four days (Figure 1 and 2A). Similarly, OP₈EO (1.03 $\mu\text{g l}^{-1}$) and OP₉EO (0.89 $\mu\text{g l}^{-1}$) oligomers predominated in settled sewage. Lower chain

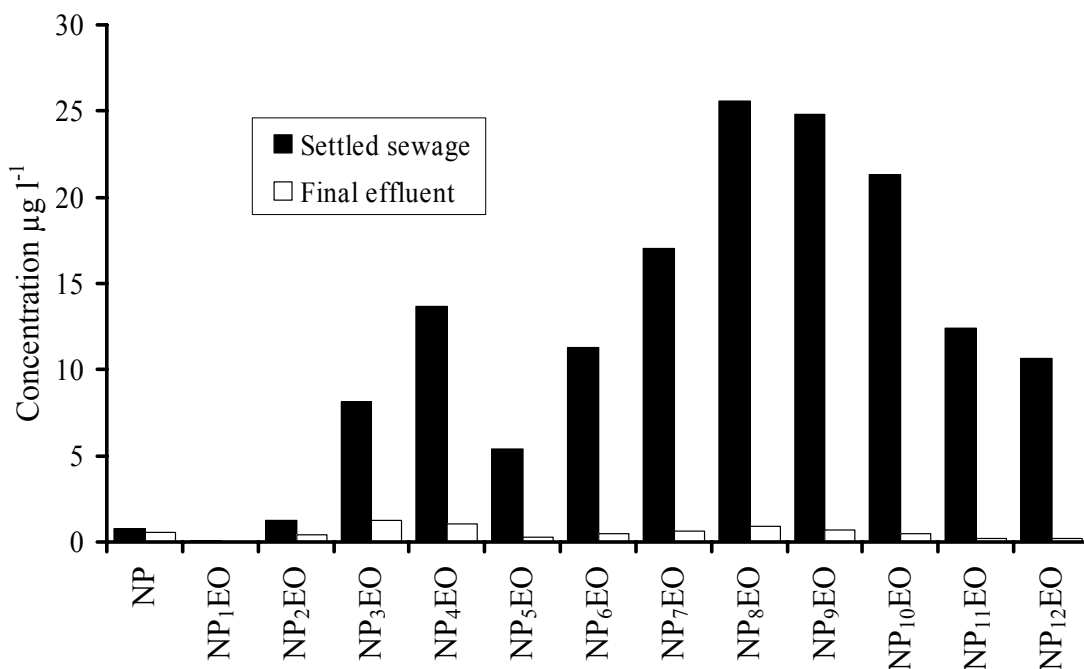


Figure 1. Average distribution, over 4 days, of NP and NPEO in settled sewage and final effluent.

ethoxylates (NP₂₋₄EO) were associated with the solid phase whereas the higher chain NPEOs were preferentially found in the aqueous phase (Figure 2A). The concentration of total NPEO (dissolved and bound fraction) entering the ASP averaged 151.5 $\mu\text{g l}^{-1}$, with the NP₇₋₉EO oligomers comprising of 59% of the total concentration, however, in the final effluent, lower chain ethoxymers NP₃EO (1.23 $\mu\text{g l}^{-1}$) and NP₄EO (1.05 $\mu\text{g l}^{-1}$) predominated (Figure 2B). These constituted approximately 35% of the total NPEO in the final effluent. Nonylphenol was observed on the first two days, but was subsequently below the detection limit. Throughout the period of sampling, concentrations of ethoxylates observed in the final effluent were 2 orders of magnitude below those present in the influent, however NP concentrations were similar to those in the influent settled sewage samples.

Compounds with $\log K_{ow}$ values > 4 , such as NP and short chain NPEOs are preferentially removed via settling of suspended solids and colloidal matter

(Langford et al. *unpublished data*). In this study, although higher ethoxylates were degraded, there was no equivalent increase in NP concentrations (or lower chain NPEO oligomers) as a result of this process, which could be attributable to either further degradation leading to complete removal of NP in the RAS/WAS or the production of intermediates, such as carboxylates, which were not determined. Carboxylated products, with the terminal ethoxy group oxidized, or dicarboxylated products, where the alkyl chain is also oxidized, have been observed

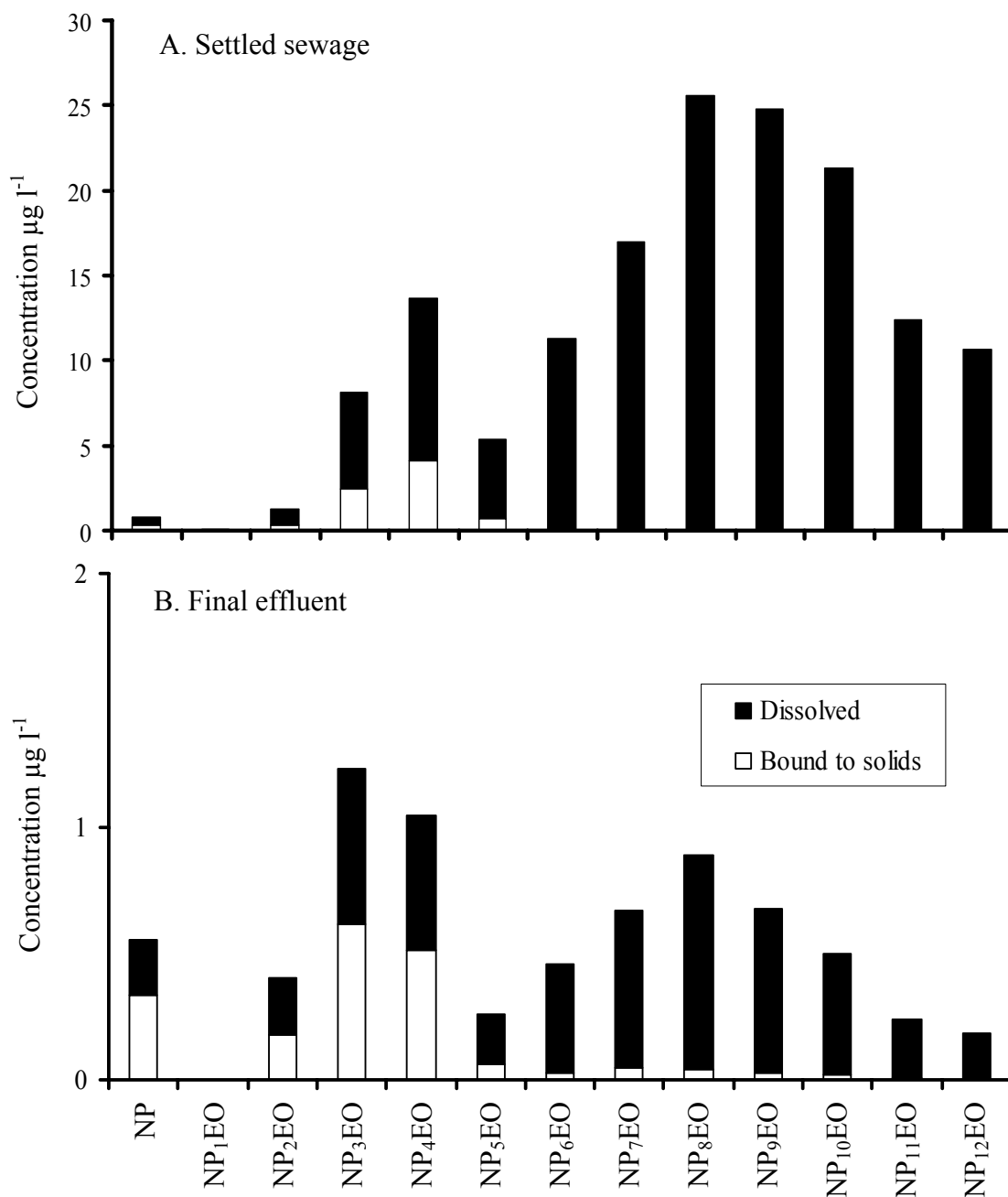


Figure 2. Average concentrations of NP and NPEO showing partitioning between the dissolved and bound fraction in settled sewage entering the ASP (A) and exiting the ASP as final effluent (B).

to be present in final effluents at concentrations above those of the residual parent NP₉EO studied (Di Corcia et al. 2000). Nonylphenol and NP₁₋₂EO, which are normally absent in the original surfactant formulations, accounted for approximately 1% of the total concentration (calculated as mass) in the settled sewage entering the ASP, therefore indicating that a proportion of the NPEOs had been biodegraded before they reached the ASP. The biotransformation observed in this work may have been facilitated by the relative abundance and availability of NP and short chain NPEOs to a consortium of acclimated microorganisms in a completely mixed aerated system as noted by others (Maki et al. 1994; Fuji et al. 2000; Corti et al. 1995).

Aerobic biodegradation of NP has been reported by Staple et al. 1999 with a half-life of 20 days in the laboratory. The sludge retention time at the works in this study was 13 days, and as such the organisms were probably well-acclimated and significant degradation of NP may therefore be more likely to occur. Nonylphenol has been observed to be almost totally removed and degraded under aerobic laboratory scale-activated sludge units at 28 °C (Tanghe et al. 1998). Although there are differences between microorganisms present in soil and sewage, comparison may be of value, and a sludge-treated soil field study showed a rapid reduction of NP, NP₁₋₂EO within the first month, however all exhibited a residual concentration after 320 days (Marcomini et al. 1989). Toxicity to microorganisms, resulting in feedback inhibition, may have resulted in the recalcitrance of the final residues of NP and NPEOs (Langford et al. 2005).

To determine the flux through the ASP, a mass balance was derived by multiplying the NPEO concentrations by the average daily flow rate. The flux of total NPEOs entering into the activated sludge process over 4 days was 1778.2 g d⁻¹ and flux out via final effluent was 77.3 g d⁻¹, indicating a high removal efficiency. The flux of the higher chain ethoxymer NP₉EO averaged 1.6 g d⁻¹. Transitory accumulation of NP₉EO was observed in WAS over all four days, which may indicate that reaction kinetics for its degradation are slower than for breakdown of the more ethoxylated oligomers. The mass influx of NP to the ASP over the four days averaged 9.1 g d⁻¹ and the flux out in the final effluent was 6.4 g d⁻¹ (a difference of 2.7 g d⁻¹) over the 4 days (Table 1). Nonylphenol has been considered to be a recalcitrant end product of the degradation of NPEOs, and due to their hydrophobic nature, it would be expected that the RAS/WAS would act as a sink for the degradation products (NP and short chain ethoxylates), however, this was not observed during this study. There was no observed accumulation of NP (or short chain NPEOs) as an end product of degradation in the sludge which would indicate further degradation of the NP, possibly to mono and dicarboxylates (Di Corcia et al. 2000).

It was evident that there was a significant reduction in the mass of NP and NPEOs once the sewage entered the activated sludge process (30% and 85% average removal percentage for NP and total NPEO respectively) (Table 1).

It has also been demonstrated that the concentration of NP and the shorter chain ethoxylates declined in the effluent following passage through the aeration tank and final clarifier. The removal of NPEOs exhibited a tendency to increase with increasing length of the ethoxylate chain. The removal efficiency of higher chain ethoxylates was more than 95% for NP₅₋₁₂EO. However, removal of lower chain ethoxylates, in particular the more recalcitrant NP₁₋₄EO was between 68 – 92%. The high SRT of 13.15 d and HRT of 0.57 d this plant operated also potentially

Table 1. Mass balance (based on 4 day average flows and concentrations) and removal efficiency between settled sewage and final effluent.

Ethoxylate	Flux (g d ⁻¹)			Removal %
	m _{in}	m _{out}	m _{in} - m _{out}	
NP	9.1	6.4	2.7	29.7
NP ₁ EO	0.5	0.1	0.4	80.0
NP ₂ EO	14.8	4.7	10.1	68.2
NP ₃ EO	95.7	14.4	81.3	85.0
NP ₄ EO	161.3	12.3	149.0	92.4
NP ₅ EO	63.2	3.1	60.1	95.1
NP ₆ EO	132.5	5.5	127.0	95.8
NP ₇ EO	199.4	7.9	191.5	96.0
NP ₈ EO	299.8	10.5	289.3	96.5
NP ₉ EO	290.9	8.0	282.9	97.2
NP ₁₀ EO	249.7	5.9	243.8	97.6
NP ₁₁ EO	145.6	2.8	142.8	98.1
NP ₁₂ EO	124.8	2.1	122.7	98.3
Σ NPEO	1778.2	77.3	1700.9	

$$\text{Removal \%} = (m_{\text{in}} - m_{\text{out}}) / m_{\text{in}} \times 100\%$$

aided the biodegradation of these compounds by an acclimatised consortium of microorganisms. The sludge wastage rate was low (180 m³ d⁻¹) meant that the acclimated microorganism could accommodate with variations in input concentrations also aiding the biodegradation of NPEO and NP. In this study, the average percentage removal of NPEOs (dissolved and solid phase) was 96% with a range from 68% - 98% in the ASP over four days. The percentage removal of NP on average was circa 30%. Percentage removal decreased with lower chain oligomers. Comparing these values with those observed in other countries, despite different STW configurations and operating conditions, the removal percentage of the NPEOs and NP was equivalent or slightly better (Table 2).

This study has observed that compounds with high sorption potential, NP and short chain NP₁₋₄EO, were preferentially removed via suspended solids from the effluent. Aerobic biodegradation also aids in their removal from the ASP where there was no evidence of accumulation or increase of NP in the ASP. The removal efficiency of higher chain ethoxylates was more than 95% for NP₅₋₁₂EO but removal of lower chain ethoxylates, in particular the more recalcitrant NP₂₋₄EO was circa 92% and less. The effluent discharge ranged from 4.7 g d⁻¹ to 12.3 g d⁻¹

for lower chain oligomers NP₂₋₄EO and NP at 6.4 g d⁻¹ on average over the four day sampling period. Since the short chain APEO oligomers and alkylphenol were associated with the solids, the lowering and removal of suspended solids from the final effluent would result in a reduction of inputs to the aquatic environment. It is also apparent that inputs of APEO are continuing despite an EU Directive restricting their marketing and use (EC 2003) and the voluntary agreement of UK industries to phase out the use of these surfactants (Eder 2004).

Table 2. Summary of the efficiency of STW in removing NPEOs.

	% removal (average)	Author	Country
NPEO	93 – 99 (97)	Naylor 1995	USA
NP	76 – 99 (94) Autumn 82 – 99 (93) Winter	Nasu et al. 2001	Japan
NP ₁₋₄ EO	86 – 99 (96) Autumn 66 – 99 (88) Winter		
NP ₅ EO	94 – 99 (98) Autumn 83 – 99 (99) Winter		
NP	93	Di Corcia and Samperi 1994	Italy
NP ₁₋₁₈ EO	84 – 98 (94)		
NP ₁₋₂₀ EO	93 – 95 (98)	Crescenzi et al. 1995	
NP	9 – 94 (65)	Ahel et al. 1994	Switzerland
NP ₁₋₂ EO	19 – 80 (50)		
NP ₃₋₂₀ EO	76 – 97 (88)		
NP	~ 30 Spring	Current study	UK
NP ₁₋₄ EO	68 – 92 (81)		
NP ₅₋₁₂ EO	95 – 98 (83)		

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