A sensitive and robust method for the determination of alkylphenol polyethoxylates and their carboxylic acids and their transformation in a trickling filter wastewater treatment plant.

Yoong K.K. Koh[1], Tze Y. Chiu[2], Alan R. Boobis[1], Elise Cartmell[2], Simon J.T. Pollard[3], Mark D. Scrimshaw[4*] and John N. Lester[2]

[1] Faculty of Medicine, Division of Experimental Medicine and Toxicology, Imperial College London, Hammersmith Campus, London, W12 ONN, UK.

[2] Centre for Water Science, School of Applied Sciences, Cranfield University, Bedfordshire, MK43 0AL, UK.

[3] Centre for Resource Efficiency and Management, Sustainable Systems Department, Cranfield University, Bedfordshire, MK43 0AL, UK.

[4] Institute for the Environment, Brunel University, Uxbridge, Middlesex, UB8 3PH, UK.

*Corresponding author: mark.scrimshaw@brunel.ac.uk, Tel.: +44(0)1895267299, Fax: +44(0)1895269761.

Abstract

This paper presents a method for the determination of alkylphenols, alkylphenol polyethoxylates (APEO) and alkylphenol ethoxycarboxylates (APEC) in the aqueous and particulate phase of wastewater samples. Quantification was achieved by liquid chromatography tandem mass spectrometry. The sensitivity of the method is demonstrated by low detection limits, in the dissolved phase 1.2 to 9.6 ng L^{-1} for alkylphenol, $AP_{1-3}EO$ and APEC and 0.1 – 4.1 ng L^{-1} for longer chain alkylphenol polyethoxylates. The method detection limit for particulate phase samples ranged from 6 to 60 ng g^{-1} for AP, $AP_{1-3}EO$ and APEC; with the longer chain APEO being from 0.5 – 20 ng g^{-1}. Matrix effects were noted in complex matrix rich samples. There was a distinct change in the distribution of alkylphenol ethoxylates during biological treatment of the wastewater, with the major biotransformation products observed being carboxylated derivatives at concentrations of up to 1768 ng L^{-1}. Shorter chain APEO were present in higher proportions in the suspended solids, due to their higher affinity to particulate matter compared to the long chain oligomers.

Keywords: Alkylphenol; polyethoxylates; carboxylates; dissolved; particulate; liquid chromatography- tandem mass spectrometry

1. Introduction

Alkylphenol polyethoxylates (APEO) are non-ionic surfactants widely used in commercial and domestic applications with a worldwide production of approximately 500 kilotons . A voluntary ban on the use of APEO in household cleaning products was introduced in northern Europe during 1995, with further restrictions on their use for industrial cleaning in 2000 (OSPAR, 2006). However, octylphenol ethoxylates (OPEO) and nonylphenol ethoxylates (NPEO) continue to be used in industrial applications, as evidenced by their continuous discharge into sewage treatment works and subsequent release into the aquatic environment .

Environmental concern in relation to these compounds results from their estrogenic activity . Biodegradation of APEO during biological wastewater treatment can occur under both aerobic and anaerobic conditions and results in the production of more persistent and estrogenic metabolites consisting of AP mono- to triethoxylates (NP_1EO, NP_2EO and NP_3EO), carboxylated intermediates and the alkylphenols (AP), nonylphenol (NP) and octylphenol (OP) . Understanding the transformation of these compounds during biological wastewater treatment processes is important for the development of strategies to control their discharge to the environment and sensitive and selective analytical methods are an essential tool for gaining the required data..

The analysis of APEO and their metabolites is a complex process due to the ethoxylated oligomers and alkyl-chain isomers which can be present . Gas chromatography (GC) and liquid chromatography (LC), are now commonly used for the determination of APEO. The use of GC for direct analysis is limited to APEO with lower numbers of ethoxy groups, while metabolic products and long chain ethoxylates require derivatisation to increase volatility. Traditionally, LC analysis with fluorescence or ultraviolet detection has been widely used. However, these techniques often lack the sensitivity and specificity required at low concentrations and LC coupled with tandem mass spectrometry (MS/MS) is now increasingly being used to determine these compounds . Most studies describe LC/MS/MS analysis of only a limited number of the possible oligomers and metabolites, focussing on either NPEO and NPECs , $AP_{1\text{-}3}EOs$ and AP , or $AP_{1\text{-}5}EOs$, AP and APEC . Furthermore, these works focus on the dissolved phase only, and the significance and distribution of these compounds on the particulate phase remains to be evaluated..

This study aims to develop a sensitive and selective analytical method to detect nonyl- and octylphenol, their ethoxylates ($NP_{1\text{-}12}EO$, $OP_{1\text{-}12}EO$) and carboxylated metabolites with up to three ethoxy units in sewage matrices. Compounds are determined in both the dissolved and particulate phases and attempts are made to achieve improvements in existing detection limits. The method was successfully applied to study a trickling filter (TF) biological wastewater treatment works, which are of particular interest due to their relatively common use. For example, a typical region in the South East of England has more TF (around 800) sites than those using the activated sludge process and few studies in the literature have reported on the fate of APEO in this process.

2. Experimental section

2.1. Reagents and chemicals

The technical 4-nonylphenol mixture of chain isomers and 4-*tert*-octylphenol were obtained from Sigma-Aldrich (Gillingham, Dorset, UK). The long-chain NPEO and OPEO were available in technical mixtures (Igepal CO210, CO520, CO720) and (Igepal CA210, CA520, CA720) respectively containing a range of oligomers (Sigma-Aldrich). Nonyl- and octyl-phenoxy acetic acid (NP_1EC, OP_1EC), 4-nonyl- and octylphenolmono- and diethoxylate ($NP_{1\text{–}2}EO$, $OP_{1\text{–}2}EO$) were obtained from QMX Laboratories (Thaxted, Essex, UK). Standards for NP_2EC, NP_3EC, OP_2EC, OP_3EC were not available commercially. Due to the absence of commercially available standards, NP_2EC and NP_3Ec were quantified with NP_1EC standard, assuming similar response factors. Similarly OP_2EC and OP_3EC were determined with OP_1EC.

Acetone, ethylacetate, acetonitrile, methanol and dichloromethane were obtained from Rathburn (Walkerburn, Scotland, UK) and acetic acid from Sigma-Aldrich. Single standard stock solutions were prepared in acetonitrile. Reagent grade MilliQ water (18.2 MΩ) (Millipore, Watford, UK) was used for spikes and preparation of solutions. The working standard solutions were prepared by further diluting the stock standard solutions with acetonitrile/MilliQ-water (50:50 v/v).

2.2. Trickling filter

The wastewater treatment works studied consisted of: primary treatment followed by a single high rate nitrifying filter with BIOdek® structured plastic media (diameter 12 m; media depth 1.8 m); two Biochemical Oxygen Demand (BOD) percolating filters with random Biofil® plastic media (diameter 12 m; media depth 1.8 m) operating in parallel; and a single tertiary trickling filter with stone media (5 m × 10 m; media

depth 4 m). This 2,800 population equivalent works treated a dry weather flow of 650 m^3 d^{-1} with the only industrial inputs of < 10 % being from a local airfield. Their use of surfactants or degreasers was limited to methyl ethyl ketone (MEK). Spot water samples of settled sewage (primary effluent) and final effluent were taken on the morning of 28th March 2007.

2.3. Analytical procedure

For determination of dissolved concentrations, settled sewage (100 mL) or final effluent (250 mL) was filtered through a 1.2 μm Whatman GF/C filter (Whatman, Maidstone, UK.). The filtered aqueous phase was then extracted using solid phase extraction (SPE) using a syringe barrel tC18 (500 mg, 3 cm^3) cartridge (Waters Ltd, Watford, UK.). The appropriate volume of sample was loaded onto the cartridges which were preconditioned with 5 mL methanol followed by 5 mL MilliQ water. The flow rate for sample extraction was kept constant between 5-10 mL min^{-1} using a vacuum manifold. When the sample had passed through, 4 mL of reagent grade water was used to rinse the cartridge, which was dried by drawing air through it for half an hour. The analytes were eluted using 10 mL ethylacetate, 10 mL dichloromethane followed by 5 mL 0.1% acetic acid in methanol. A rotary evaporator (Heidolph Instruments, Schwabach, Germany) was employed to concentrate the extracts to 1 mL which was then evaporated to complete dryness under a gentle stream of nitrogen. The extract was reconstituted with 0.25 mL acetonitrile/MilliQ-H_2O (50:50 v/v) and transferred to an autosampler vial prior to analysis using LC/MS/MS.

The determination of alkylphenolic compounds in the particulate phase was also performed in this study. The filter papers with suspended solids were freeze-dried, shredded and subsequently extracted using 10 mL methanol/acetone (1:1) by shaking on a Multi-Reax system (Heidolph UK, Germany) for half an hour in a 25 mL Teflon tube. The solids were separated by centrifugation at 1500 g for 10 minutes and the supernatant decanted off. This procedure was repeated twice and the combined extracts (20 mL) were then cleaned by passing through a 500 mg and 3 cm^3 silica SPE cartridge (Waters Ltd, Watford, UK) and eluted using 10% acetic acid in 10 mL methanol prior to drying, reconstitution with 0.25 mL acetonitrile/MQ-H_2O (50:50 v/v) and quantification by LC/MS/MS.

2.4. LC/MS/MS analysis

Analytes were determined using LC/ESI/MS/MS consisting of an HPLC (Waters Alliance HPLC system 2695) coupled to a Waters Quattro Premier XE mass spectrometer with a Z-Spray ESI source (Micromass, Manchester, UK.). The AP, APEC and APEO were separated on a Gemini C18 column (3 µm particle size, 100 mm × 2 mm i.d., Phenomenex, Macclesfield, UK.). The mass spectrometer was operated in the negative electrospray ionisation (ESI^-) (AP and APEC) or positive electrospray mode (ESI^+) (APEO) using multiple reaction monitoring (MRM). Nitrogen was used as the nebuliser gas and argon as the collision gas. The conditions for detection by the mass spectrometer were as follows, capillary voltage, 3.20 kV in the positive mode and -2.3 kV in the negative mode, extractor lens at 3.0 V, RF lens at 0.5 V in the positive mode and 1.0 V in the negative mode, multiplier voltage, 650 V, desolvation gas flow, 1000 L h^{-1}, cone voltage as shown in Table 1, cone gas flow at 50 L h^{-1}, desolvation temperature at 350 °C and source temperature at 120 °C.

Two separate chromatographic runs were used, one for the separation of $AP_{3\text{-}12}EO$; and the other for $AP_{1\text{-}2}EO$, APEC and APs. In both cases, separation was achieved using MQ water containing 20 mM NH_4OH (solvent A) and acetonitrile containing 20 mM NH_4OH (solvent B). The use of ammonia results in formation of the NH_4^+ adduct ions by the APEO, rather than more stable sodium adducts, which facilitates fragmentation of parent ions in the collision cell of the MS/MS (Table 1). The gradient conditions for $AP_{3\text{-}12}EO$ were; time zero, 45 % solvent B (5 min) followed by a linear increased in gradient to 80 % solvent B which was maintained for 40 min. Following this gradient, the conditions were maintained at 90 % solvent B for 5 min before the column was re-equilibrated to starting conditions at 20 % solvent B. The total run time was 50 min and A sample volume of 10 µL was injected at a flow rate of 0.2 mL min^{-1}.

Conditions for the $AP_{1\text{-}2}EO$, APEC and APs started with 20 % solvent B increasing to 45 % over 10 min (Fig. 1). This was followed by a linear increased to 80 % solvent B which was maintained for 20 minutes. Following the increase gradient from 80 to 90 % solvent B, the conditions were maintained at 90 % solvent B for 5 min before the column was re-equilibrated to starting conditions at 20 % solvent B for 10 min prior to next run. Similarly, the total run time for this analysis was 50 min with 10 µL sample volume injected and flow rate was kept at 0.2 mL min^{-1}.

Fig. 1. Chromatograms of alkyphenolic compounds. Standard concentrations 0.5 mg L^{-1} (only one product ion for each alkylphenolic oligomer is shown).

3. Results and Discussion

3.1 Optimisation of LC and MS/MS conditions

Identification of the alkylphenolic compounds was ensured by monitoring two characteristic precursor-product ion transitions and obtaining a retention time match. For quantification, the most sensitive transition was used. However, some compounds produced only one sensitive product ion (Table 1). Quantification using external calibration with standard solution mixtures was used. In all instances the calibration curves exhibited $r^2 \geq 0.99$ for all compounds.

The APEO exhibit a high affinity for alkali metal ions resulting in the formation of sodium adducts $[M + Na]^+$ rather than the protonated molecules in unmodified mobile phases . However, the sodium adducts tend to be stable and do not fragment in the collision cell, and the ammonium adducts have been favoured for MS/MS . In this study, the use of ammonium adducts for MRM detection improved the detection limits due to the high degree of fragmentation in the collision cell.

3.2 Evaluation of method performance

The recoveries and relative standard deviations were determined in experiments where the analytes in the dissolved phase were in the concentration range of 0.1 μg L^{-1} (low spike) and 1 μg L^{-1} (high spike) using MQ water and wastewater samples (n = 3). Filtration of the wastewater samples was undertaken prior to SPE to remove particles. Recoveries in MQ water (low and high spiking levels) ranged from 71% to 98% for AP, APEO and APEC. Generally, the relative standard deviations (RSDs) were in the range of 2 to 7 %. Typically, recoveries for settled sewage (low and high spiking levels) ranged from 62% to 98% for AP, APEO and APEC. Relative standard deviations obtained were 2 to 8 % for these alkylphenolic compounds. Recovery values and RSD were similar for spiked (low and high) final effluent samples for all alkylphenolic compounds.

The recoveries and relative standard deviations were also determined for particulate samples where the analytes were in the concentration range of 0.125 μg g^{-1} (low spike) and 1.25 μg g^{-1} (high spike) using suspended solids samples (n=3) and sludge samples (n=3). The total suspended solids were 437.5 mg L^{-1} and 23.5 mg L^{-1} for settled sewage and final effluent samples. Recoveries for APEO sorbed to the particulate phased ranged from 51% to 105% for AP, APEO and APEC for low and high spikes

Table 1. Characteristic LC/MS/MS parameters and detection limits of alkylphenol polyethoxylates (NPEO and OPEO). Recoveries and matrix suppression for determination of the dissolved phase in final effluent samples.

Compounds	m/z precursor $[M + NH_4]^+$	Cone (V)	Product ions (Collision potential)	Retention time (min)	IDL (pg)	Recoveries % (RSD)	Matrix suppression (%)	MDL (ng L^{-1})	MDL (ng g^{-1})
NP_3EO	370	40	[a]353(10), 226.5(20)	27.87	0.2	84(2)	10	0.1	0.5
NP_4EO	414	30	[a]397(14), 270(20)	27.71	4.7	91(5)	8	1.5	7.5
NP_5EO	458	30	[a]440(20), 315(25)	27.63	7.9	85(2)	9	2.1	10
NP_6EO	502	30	[a]485(20), 359(30)	27.47	13.4	90(2)	8	2.7	13
NP_7EO	546	30	[a]529(23), 403(25)	27.23	8.3	90(3)	8	0.8	4
NP_8EO	590	30	[a]573(25), 447(27)	27.07	3.5	90(3)	8	0.7	4
NP_9EO	634	30	[a]617(25), 335(30)	26.82	2.9	88(2)	9	2.2	11
$NP_{10}EO$	678	30	[a]661(25), 132.5(40)	26.66	3.0	98(3)	8	2.1	10
$NP_{11}EO$	722	30	[a]705(25), 291(40)	26.42	1.9	89(3)	8	0.9	4.5
$NP_{12}EO$	766	30	[a]749(30), 291(35)	26.26	1.0	90(2)	9	1.1	5.5
OP_3EO	356.5	20	[a]338.5(10), 227(10)	22.84	4.9	80(5)	11	1.8	9
OP_4EO	400.5	20	[a]382.5(15), 270.5(15)	22.84	5.9	85(3)	10	4.1	20
OP_5EO	444	20	[a]426.5(10),315(10)	22.76	0.2	80(3)	9	1.4	7
OP_6EO	488	30	[a]471(15), 359(20)	22.68	6.0	79(6)	9	3.0	15
OP_7EO	532	30	[a]515(20), 403(20)	22.60	8.6	79(9)	8	0.6	3
OP_8EO	576	30	[a]559(20), 447(25)	22.43	7.9	93(3)	8	1.3	6.5
OP_9EO	620	30	[a]603(20), 491(25)	22.27	5.5	85(4)	9	2.0	10
$OP_{10}EO$	664	30	[a]647(20), 535(30)	22.11	3.0	90(7)	8	0.8	4
$OP_{11}EO$	708	30	[a]690(20), 579(30)	22.03	0.1	88(9)	8	0.1	0.5
$OP_{12}EO$	752	30	[a]735(20), 623(30)	21.87	0.1	82(2)	9	0.5	2.5
[b]NP_1EO	282	25	[a]127(25), 265(25)	29.11	40	86(2)	10	1.2	6
[b]NP_2EO	326	40	[a]183(40), 121(40)	28.88	7	73(2)	11	2.5	12
[c]NP_1EC	263.5	20	[a]205(20), 106(30)	11.32	3	94(3)	9	2.5	12
[c]NP_2EC	307	20	205(20)	11.32	N/C			N/C	N/C
[c]NP_3EC	351	20	205(20)	11.32	N/C			N/C	N/C
[b]OP_1EO	268	15	[a]113(10), 250(20)	25.92	58	81(3)	10	9.6	48
[b]OP_2EO	312	20	[a]183(10), 295(10)	25.73	5	73(2)	11	5.1	25
[c]OP_1EC	277.5	20	[a]219(18), 133(40)	10.87	3	97(6)	8	12	60
[c]OP_2EC	321	20	219(18)	10.87	N/C			N/C	N/C
[c]OP_3EC	365	20	219(18)	10.87	N/C			N/C	N/C
[c]NP	219	30	[a]132.5(30), 147(35)	29.43	5	73(2)	11	2.3	11
[c]OP	205.5	30	[a]134(25), 106(20)	26.32	4	72(1)	9	6.9	34

[a]MRM used for quantification; [b]$[M + NH_4]^+$; [c]$[M - H]^-$; IDL were calculated by a *S/N* of 3 from the MRM chromatograms of the standard solution mixture
MDL for dissolved phase from spiked MQ (0.1 µg L^{-1}) in 250 mL reagent water; MDL for the particulate phase from 0.125 µg g^{-1} in 0.2 g sludge dry weight
N/C: Not calculated (no unique standards available).

and their corresponding RSDs were in the range of 1 to 14. Recoveries in spiked sludge ranged from 51 % to 104 % for AP, APEO and APEC and their RSDs ranged from 1 to 8. This methodology demonstrates that consistent recovery values and low RSD could be obtained from different matrices.

The method detection limit (MDL) was determined by subjecting the entire analytical extraction and detection procedure to the determination of spiked reagent water, settled sewage and final effluent samples in both the dissolved and particulate phases. The MDL determined for the entire SPE/LC/MS/MS ranged from 1.2 to 9.6 ng L^{-1} for (AP, APEO and APEC) and from 0.1 – 4 ng L^{-1} for long chain APEO (Table 1). The direct comparison of MDL with values reported by other authors is difficult due to the use of a range of sample matrices, the number of analytes and the lack of information on how the limits have been derived. In comparison to methods that allow the determination of selected APEO, APEC and AP in aqueous samples, the sensitivity of the method presented here is generally greater than other studies, however, the work by Jahnke et al. (2004) appears particularly sensitive (Table 2). These authors utilised greater sample volumes (1 L) than in this study, and made their samples to a final volume of (100 μL) overall ten times greater concentration factor than in this study, possibly accounting for much of the difference.

The detection limits for compounds on the particulate phase (removed by filtration with the GF/C filters) were calculated based on determination of ~0.2 g of total suspended solids. The MDL determined for the entire SPE/LC/MS/MS ranged from 6 to 60 ng g^{-1} for AP, short chain APEO and APEC and 0.5 – 20 ng g^{-1} for long chain APEO.

3.3 Matrix effects

A common problem when analysing APEOs and their degradation products in wastewater samples by MS is the suppression of the analyte signal as a result of co-eluting compounds impairing ionisation in the ESI source. As a result, LC/MS/MS signals of external standard samples, usually prepared in pure solvent, may be enhanced in relation to those from samples prepared from an environmental matrix .

Table 2. Comparison of method detection limit (ng L^{-1}) with other publications.

Analyte	MDL (this study)	MDL [a]	MDL [b]	MDL [c]	MDL [d]	MDL [e]	MDL [f]
NP_1EO	1.2	10	100	14.4	100	50	15
NP_2EO	2.5	0.2	40	13	100	20	6
NP_1EC	2.5	0.1	20	86.7	1	10	13
OP_1EO	9.6	12	100	10	100	-	-
OP_2EO	5.1	0.1	40	3.3	100	-	-
OP_1EC	12	0.04	20	-	1	-	-
NP	2.3	2.0	20	8.4	10	-	11
OP	6.9	4.0	20	6.1	5	-	-

[a, d, e] $[APEO + NH_4]^+$ adducts and $[M - H]^-$ ions (AP and APEC) monitored using LC/ESI/MS/MS.
[b, f] $[APEO + NH_4]^+$ adducts and $[M - H]^-$ ions (AP and APEC) monitored using LC/ESI/MS.
[c] GC/MS after derivatization.

To evaluate and quantify the impact of matrix effects on signal intensity, the methodology was applied to water, settled sewage and effluent matrices which were unspiked or spiked with the alkylphenolic compounds (low and high spike of 0.1 µg L^{-1} and 1 µg L^{-1} respectively). The relative signal suppression was derived using equation (1) and the result expressed as a percentage:

$$\frac{A_s - (A_{sp} - A_{usp})}{A_s} \times 100\% \qquad (1)$$

where A_s is the peak area of the analyte in pure standard solution, A_{sp} is the peak area in the spiked matrix extract and A_{usp} is the peak area in the unspiked matrix extract.

Matrix effects resulted in a suppression of the analyte signal of 8-24 % in the $AP_{1-12}EO$ (dissolved phase), with greater matrix effects observed in the settle sewage of 12-24 % compared to the final effluent at 8-11 % (dissolved phase). Significantly higher matrix interference was observed for solid samples of up to 27 % in sewage sludge for $AP_{1-12}EO$ and up to 24 % for settled sewage for $AP_{1-2}EC$ and APs. The matrix effect can be reduced by a careful and selective extraction procedure and sample clean-up and the addition of mobile phase additives . Such approaches, along with a smaller injection volume (10 µL) and longer analysis time (~50 min) to give well separated chromatography, were incorporated within this study to minimise matrix effects. Overall matrix effects on the signal were less than those observed by Jahnke et al. (2004), who reported suppression from 32 – 79 %, and commented that "for high enrichment factors, matrix interferences are distinct", and it is apparent that

there is a trade-off in utilising high enrichment to increase sensitivity against uncertainty resulting from matrix effects and that matrix effects are sample dependent.

3.4 Concentration of alkylphenolic compounds in a trickling filter works

The method was applied to evaluate the transformation of the APEO during biological treatment through analysis of settled sewage and final effluent at a trickling filter wastewater treatment plant. There was a change in the distribution of the NPEO oligomers during treatment (Fig. 2) which was also observed for the OPEO (data not shown). In the settled sewage, the higher ethoxylates ($NP_{4\text{-}12}EO$) accounted for 91 % of the nonylphenol compounds, however, following biological treatment, the distribution of both nonyl- and octylphenol compounds changed and the carboxylated metabolites ($NP_{1\text{-}3}EC$) were predominant amongst the nonylphenol compounds (58 %) and increased in the octylphenols (20 %). Taking into account the presence of the carboxylated intermediates, 64 % of the nonylphenol compounds and 86 % of the octylphenols passed through treatment to the final effluent. These results support observations by other workers who have determined carboxylated intermediates, and demonstrates their significance in discharges (Jahnke et al., 2004: Loyo-Rosales et al., 2007) and that there was a shift in the distribution of oligomers due to cleavage of ethoxy units and carboxylation through the biological treatment process.

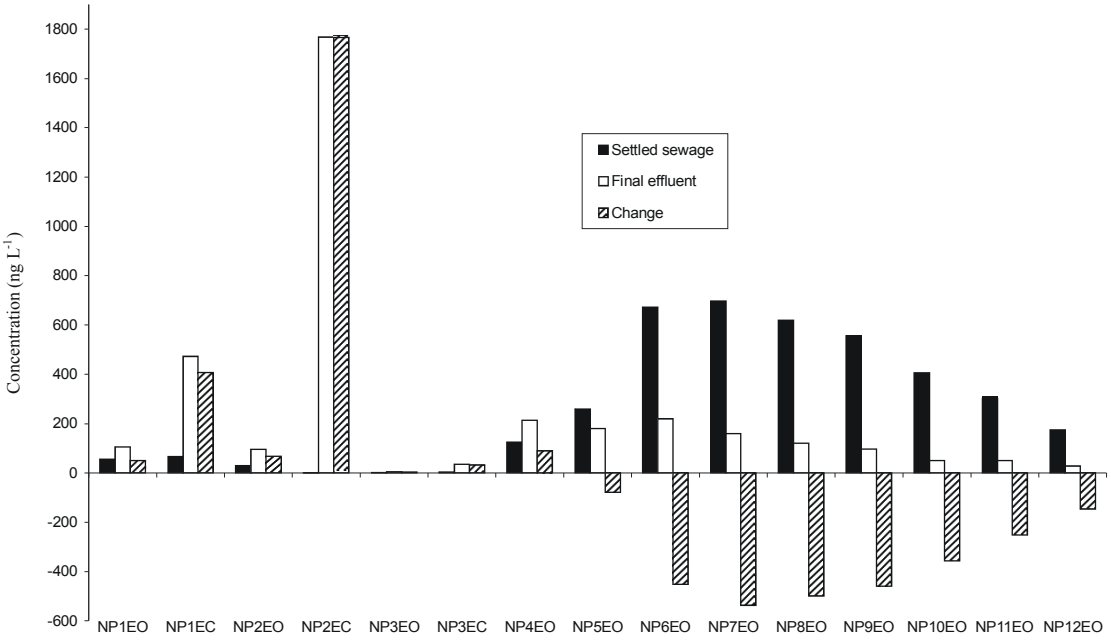

Fig. 2. Concentrations of the NPEO and $NP_{1\text{-}3}EC$ in settled sewage and final effluent with the change in concentrations during the process highlighted.

The major biotransformation products of the NPEO, $NP_{1-3}EC$, formed during the wastewater treatment, were present in the final effluent at relatively high concentrations (35 – 1768 ng L^{-1}) as a result of biodegradation. As demonstrated in this study progressive shortening of the ethoxylate chain occurs during the biological stage of wastewater treatment, although this is not efficient enough to remove all of the higher oligomers ($AP_{4-12}EO$) which were still observed in the final effluent. This study corroborated the findings of others cited in this manuscript in that APEO do degrade, however, estrogenic carboxylated metabolites were generated in the process. In this study, the NP_2EC predominated in the effluent (Fig. 2), a characteristic also observed in activated sludge processes, though these metabolites are more effectively removed in membrane reactors (González et al., 2007)

The determination of these compounds on the particulate phase demonstrates the significance of sorption to solids, with 68 % of the hydrophobic NP (and 50 % of the OP) being associated with the suspended solids (Fig. 3). Suspended solids in the final effluent at the treatment plant were 23.5 mg L^{-1}. Although at this site the final effluent concentration of nonylphenol would meet the PNEC value set by the UK Environment Agency (330 ng L^{-1}), it would fail for octylphenol (6 ng L^{-1}). Whilst there are as yet no environmental quality standards for the carboxylated intermediates these compounds appear to be discharged at relatively high concentrations and their solubility will mean the ability to remove them from effluents with conventional biological treatment is likely to be challenging.

4. Conclusions

An analytical method for alkylphenolic compounds has been developed, consisting of a SPE extraction (dissolved phase); and solvent extraction with silica clean-up (particulate phase) followed by LC/MS/MS detection. Evaluation of the methodology indicates the importance of considering matrix effects, in particular if concentration factors are increased to give lower detection limits and more complex, or “dirty” samples are analysed. The results of determining the transformation of the compounds during biological wastewater treatment demonstrate the importance of determining not only the alkylphenols and ethoxylates, but also the APEC intermediates, as the highest concentrations found in the final effluent were for NP_1EC and NP_2EC.

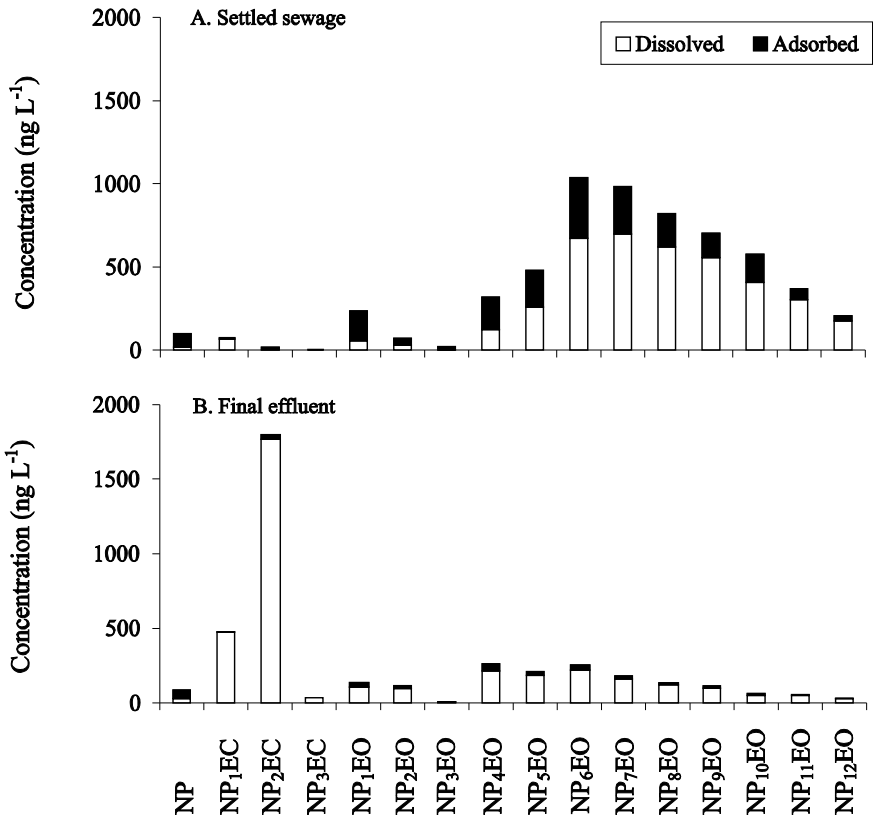

Fig. 3. Concentrations of NP, $NP_{1-3}EC$ and $NP_{1-12}EO$ showing partitioning between the dissolved and particulate fraction in settled sewage (A) and final effluent (B).

Acknowledgements

One of the authors (Y.K.K. Koh) is grateful to the Public Utilities Board of Singapore for the award of a PhD scholarship. The authors would like to thank the following companies: Anglian Water Plc, Severn Trent Plc, Thames Water, United Utilities Plc and Yorkshire Water (Kelda Group Plc) for providing their support and funding.

References