Chemosphere 73 (2008) 551–556

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

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 [a], Tze Y. Chiu [b], Alan R. Boobis [a], Elise Cartmell [b], Simon J.T. Pollard [c], Mark D. Scrimshaw [d,*], John N. Lester [b]

[a] Faculty of Medicine, Division of Experimental Medicine and Toxicology, Imperial College London, Hammersmith Campus, London W12 ONN, UK
[b] Centre for Water Science, School of Applied Sciences, Cranfield University, Bedfordshire MK43 0AL, UK
[c] Centre for Resource Efficiency and Management, Sustainable Systems Department, Cranfield University, Bedfordshire MK43 0AL, UK
[d] Institute for the Environment, Brunel University, Uxbridge, Middlesex UB8 3PH, UK

ARTICLE INFO

Article history:
Received 22 January 2008
Received in revised form 5 June 2008
Accepted 5 June 2008
Available online 25 July 2008

Keywords:
Alkylphenol
Polyethoxylates
Carboxylates
Dissolved
Particulate

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 1.2–9.6 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 to 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.

1. Introduction

Alkylphenol polyethoxylates (APEO) are non-ionic surfactants widely used in commercial and domestic applications with a worldwide production of approximately 500 kilotons (Petrovic and Barceló, 2001; Langford and Lester, 2002). 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 (Cheng et al., 2006; Cailleaud et al., 2007).

Environmental concern in relation to these compounds results from their estrogenic activity (Routledge and Sumpter, 1996). 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) (González et al., 2007; Langford et al., 2007; Petrovic et al., 2007). 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 (Scrimshaw et al., 2004). 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 derivatization to increase volatility. Traditionally, LC analysis with fluorescence (Ahel et al., 2000; Marcomini et al., 2000) or ultraviolet (Zhou et al., 1990) 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 (Loyo-Rosales et al., 2003, 2007; Petrovic et al., 2003). Most studies describe LC/MS/MS analysis of only a limited number of the possible oligomers and metabolites, focussing on either NPEO and NPECs

* Corresponding author. Tel.: +44 (0)1895267299; fax: +44 (0)1895269761.
E-mail address: mark.scrimshaw@brunel.ac.uk (M.D. Scrimshaw).

0045-6535/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2008.06.016

(Houde et al., 2002; Petrovic et al., 2003), $AP_{1-3}EOs$ and AP (Ferguson et al., 2001), or $AP_{1-5}EOs$, AP and APEC (Loyo-Rosales et al., 2003; Loos et al., 2007). 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 ($NP_{1-12}EO$, $OP_{1-12}EO$) 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 ($NP_{1-2}EO$, $OP_{1-2}EO$) were obtained from QMX Laboratories (Thaxted, Essex, UK). Standards for NP_2EC, NP_3EC, OP_2EC and 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 2800 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 and 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-water (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 min 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/MilliQ-water (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 $AP_{3-12}EO$; and the other for $AP_{1-2}EO$, APEC and APs. In both cases, separation was achieved using MilliQ-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 $AP_{3-12}EO$ 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 $AP_{1-2}EO$, APEC and APs started with 20% solvent B increasing to 45% over 10 min (Fig. 1). This was followed by