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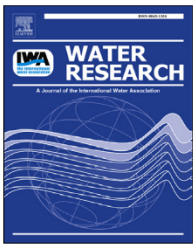

Assessing potential modifications to the activated sludge process to improve simultaneous removal of a diverse range of micropollutants

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ABSTRACT

It is proposed that wastewater treatment facilities meet legislated discharge limits for a range of micropollutants. However, the heterogeneity of these micropollutants in wastewaters make removal difficult to predict since their chemistry is so diverse. In this study, a range of organic and inorganic micropollutants known to be preferentially removed via different mechanisms were selected to challenge the activated sludge process (ASP) and determine its potential to achieve simultaneous micropollutant removal. At a fixed hydraulic retention time (HRT) of 8 h, the influence of an increase in solids retention time (SRT) on removal was evaluated. Maximum achievable micropollutant removal was recorded for all chemicals (estrogens, nonylphenolics and metals) at the highest SRT studied (27 days). Also, optimisation of HRT by extension to 24 h further augmented organic biodegradation. Most notable was the enhancement in removal of the considerably recalcitrant synthetic estrogen 17α-ethinylestradiol which increased to 65 ± 19%. Regression analysis indicates that this enhanced micropollutant behaviour is ostensibly related to the concomitant reduction in food: microorganism ratio. Interestingly, extended HRT also initiated nonylphenol biodegradation which has not been consistently observed previously in real wastewaters. However, extending HRT increased the solubilisation of particulate bound metals, increasing effluent aqueous metals concentrations (i.e., 0.45 μm filtered) by >100%. This is significant as only the aqueous metal phase is to be considered for environmental compliance. Consequently, identification of an optimum process condition for generic micropollutant removal is expected to favour a more integrated approach where upstream process unit optimisation (i.e., primary sedimentation) is demanded to reduce loading of the particle bound metal phase onto the ASP, thereby enabling longer HRT in the ASP to be considered for optimum removal of organic micropollutants.

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1. Introduction

Current operations of secondary wastewater treatment works (WwTWs) do not enable compliance to Environmental Quality Standards (EQSs) and proposed legislative targets for a wide variety of micropollutants (Gardner et al., 2012). However, the environmental and economic cost of implementing and operating advanced processes underpins the need to optimise these secondary processes (Jones et al., 2007). Activated sludge is a widely used secondary process with a proven ability to remove a variety of micropollutants (Clara et al., 2005; Koh et al., 2009; Radjenovic et al., 2009; McAdam et al., 2010, 2011; Petrie et al., 2013a). Micropollutant removal by activated sludge can be attributed mostly to sorption and biodegradation mechanisms (Joss et al., 2004; Andersen et al., 2005; Langford et al., 2005; Radjenovic et al., 2009; Petrie et al., 2013a). Therefore measuring process performance and optimising operation is needed on micropollutants which exhibit differing susceptibility to these mechanisms for removal. Natural and synthetic steroid estrogens, nonylphenolic surfactants (NPx) and metals encompass such diversity. These are describable as biodegradable and refractory organics (Joss et al., 2004; Andersen et al., 2005; Clara et al., 2005; Petrie et al., 2013b, submitted for publication) and non-biodegradable inorganics (Santos et al., 2010), respectively. These exhibit a broad range of physico-chemical properties (Table S1) which contributes to their differing fate and behaviour during wastewater treatment. For example, hydrophobicity (log K_{ow}) can be used as a reasonable predictor for sorption behaviour of estrogens (Petrie et al., 2014). Nevertheless, biodegradation is accepted as their major removal pathway (Andersen et al., 2005; Petrie et al., 2014). The removal of NPx is more complex as this family of chemicals possess a variety of physico-chemical properties and breakdown pathways. The relatively hydrophilic long chained NPx's are highly susceptible to biological attack (McAdam et al., 2011). However, their biotransformation can result in the formation of shorter chained intermediates and NP (Petrie et al., 2013b). These chemicals have log K_{ow}'s > 5 (Table S1) and a relatively high propensity to sludge partitioning is expected (Byrns, 2001). In contrast, metals rely entirely on partitioning within the activated sludge matrix for removal. This is considered to be driven by three major processes which are; physical entrapment of insoluble metals, binding of soluble metals to bacterial walls and extracellular polymers, and active cellular uptake by bacterial cells (Ziolko et al., 2011).

Micropollutant removal is considered to be influenced by activated sludge process variables, solids retention time (SRT) and hydraulic retention time (HRT) (Svenson et al., 2003; Clara et al., 2005; Johnson et al., 2005; Hamid and Eskicioglu, 2012; Maeng et al., 2013). Despite a large volume of research undertaken on this subject area, it remains unclear whether operation can be optimised to achieve maximum removals of all micropollutants simultaneously. To illustrate, an extended SRT (≥10 days) is considered necessary to augment removal of those biodegradable micropollutants (Clara et al., 2005; McAdam et al., 2010). This is thought to enable the enrichment of a more diverse bacterial community more capable of organic micropollutant biodegradation. However, such conditions are hypothesised to be detrimental to metals removal (Santos et al., 2010). It is anticipated that metals are solubilised by chelators produced by biomasses at SRTs >10 days and are therefore not available for removal within the activated sludge matrix (Santos et al., 2010). Furthermore, there is a paucity of information on the impact of HRT to micropollutant removal. It can be postulated that longer HRTs (and contact time) enable greater biodegradation of the organic micropollutants. Previous research in this field has traditionally focussed on the broad comparison of different full-scale activated sludge plants (ASPs) operating at various SRT and HRTs (Svenson et al., 2003; Clara et al., 2005; Johnson et al., 2005; Koh et al., 2009; McAdam et al., 2010, 2011). However, full-scale processes tend to suffer from poor process control resulting in a dynamic system with considerable variation in both SRT and HRT. To demonstrate, a full-scale ASP designed for operation at 10 day SRT and 8 h HRT was subject to influent flow variations of between 50 and 600 $m^3\ h^{-1}$ (Aboobakar et al., 2013) which, based on a simple solids mass balance, implies an estimated SRT range of between 4 and 20 days. Consequently, to overcome the limitations of dynamic process conditions observed at full scale, this study utilised a pilot-scale ASP to enable good process control and circumvent variations in receiving sewage composition and flow. This allowed SRT and HRT to be focused on separately and their individual impact to micropollutant removal to be assessed. The pilot-scale ASP was operated at: (i) 3, 10 and 27 day SRTs whilst at a constant HRT (8 h) and (ii) 8, 16 and 24 h HRTs at a constant SRT (27 days). To measure ASP performance for the removal of a wide range of micropollutants of varied chemistry and preferred removal mechanisms under this range of operating conditions - steroid estrogens, NPx's and metals were monitored. To our knowledge this is the first study which has closely controlled ASP operation and measured the impact to the removal of such a diverse range of chemicals simultaneously in real wastewater.

2. Materials and methods

2.1. Chemicals

Estrogen standards (>98% purity); estrone (E1), 17β-estradiol (E2), estriol (E3), 17α-ethinylestradiol (EE2) and estrone sulphate (E1-3S) were purchased from Sigma Aldrich (Dorset, UK). Deuterated internal standards; E1-d_4, E2-d_5, E3-d_3, EE2-d_4 and E1-3S-d_4 sulfate were obtained from QMX Laboratories (Thaxted, UK). Technical 4-NP, 4-nonylphenol-monoethoxylate (NP_1EO), the diethoxylate compound (NP_2EO) and the longer chain NPEOs ($NP_{3\text{-}12}EO$) were purchased from Sigma Aldrich (Dorset, UK). Long chain NPEOs were purchased as the technical mixtures CO210, CO520 and CO720. Nonylphenoxy acetic acid (NP_1EC) was obtained from QMX laboratories (Thaxted, UK). Single element metal solutions of zinc (Zn), copper (Cu), lead (Pb), nickel (Ni), cadmium (Cd) and rhodium (internal standard), and OPTIMA trace metal grade nitric acid were obtained from Fisher Scientific (Leicestershire, UK). The high performance liquid chromatography grade solvents; acetone, methanol, dichloromethane, ethylacetate and hexane were purchased from Rathburn Chemicals

(Walkerburn, UK). Ammonium hydroxide was ACS grade and obtained from Sigma Aldrich (Dorset, UK) and ultra-pure water of 18.2 MΩ quality (Elga, Marlow, UK) was used in the preparation of mobile phases. Chemical oxygen demand (COD), ammoniacal nitrogen, nitrate, nitrite and total nitrogen proprietary cell test kits were purchased from VWR International (Leicestershire, UK).

2.2. Pilot plant operation

A pilot-scale ASP was sited at a WwTWs in the east of England (3000 population equivalent) and consisted of a 0.18 m^3 primary sedimentation tank, a 0.36 m^3 aerated basin and a 0.10 m^3 final clarifier (Petrie et al., 2014) (Fig. 1). The plant was seeded with biomass from a full-scale ASP (280,000 population equivalent) and operated with municipal crude sewage containing indigenous concentrations of all micropollutants (Petrie et al., 2013b, 2013c). Less than 5% of the crude sewage was attributed to industrial inputs from a local airfield. The influent flow rate of the pilot plant was controlled to achieve a constant HRT. Return activated sludge (RAS) was 0.55 of the influent flow in all studies. Solids retention time was controlled by daily wastage of sludge (waste activated sludge, WAS) from the base of the final clarifier following correction for loss of effluent suspended solids. The system was operated for at least three SRTs prior to monitoring at each different condition to ensure continuity of SRT and HRT performance was established. Once under steady state conditions, sampling was undertaken over seven consecutive days (Monday to Sunday) at varying times between 7:00 and 19:00 h. Samples (~10 L – to obtain suitable quantities of suspended solids for particulate analysis) were collected in 2.5 L borosilicate glass vessels with Teflon lined caps for the determination of organic micropollutants and sanitary determinands. Samples for metals analysis were collected using acid washed 50 ml plastic centrifuge tubes. Grab sampling was employed due to the well-known problems of chemical stability whilst using composite samplers (Baker and Kasprzyk-Hordern, 2013). Upon collection, samples were transported to the laboratory (within 10 min) and processed immediately. During the seven day sampling campaigns which were performed over a two year period, no significant rainfall was experienced. The impact of SRT was investigated by maintaining a constant 8 h HRT over the secondary treatment stage. Solid retention times of 3, 10 and 27 days were assessed. In HRT studies the SRT was maintained at 27 days and HRTs of 8, 16 and 24 h examined.

2.3. Sanitary determine and analysis

Chemical oxygen demand, soluble COD, ammoniacal nitrogen, nitrate, nitrite and total nitrogen were determined using proprietary cell test kits (VWR International, Leicestershire, UK) and subsequent detection by spectrophotometry. The aqueous phase of wastewater was obtained by filtration through a 1.2 μm filter (Whatman, Maidstone, UK). Suspended solids, volatile suspended solids (VSS) and biochemical oxygen demand (BOD) were determined using standard methods (APHA, 1998). Biomass of each operational condition was characterised by separating extracellular polymeric substance (EPS) and soluble micro products fractions (Le-Clech et al., 2006). Briefly, 50 ml of biomass was centrifuged at 1500× *g* for 5 min and the supernatant filtered (0.2 μm) to obtain the SMP fraction. The solid fraction was re-suspended in 50 ml of deionised water and placed in an oven at 105 °C for 1 h. The extracted EPS was obtained by allowing the sample to cool to room temperature, centrifuging at 1500× *g* for 5 min and filtering (0.2 μm). Protein and carbohydrate concentrations were quantified using the phenol–sulphuric acid method (Zhang et al., 1999) and modified Lowry method (Frølund et al., 1995) respectively. Charge (as zeta potential) was determined using a Zetasizer 2000 (Malvern Instruments LTD, Worcestershire, UK) whereas activated sludge floc size was measured using a Mastersizer 2000 (Malvern Instruments LTD, Worcestershire, UK).

2.4. Micropollutant analysis

2.4.1. Steroid estrogens

For all samples collected (settled sewage, RAS/WAS and final effluent), aqueous and particulate phases were separated by centrifugation and filtration (Petrie et al., 2013c). The separated aqueous and particulate phases were then analysed separately using a three stage extraction and clean up procedure. Quantification was by ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) as previously described (Petrie et al., 2013c). A triple quadrupole was utilised and two multiple reaction monitoring (MRM) transitions were monitored per compound. Deuterated surrogates were used to compensate for any loss of analytes during sample preparation. The method achieved aqueous and particulate method detection limits (MDLs) of 5.0×10^{-5} to 1.7×10^{-4} μg l^{-1} and 2.5×10^{-3} to 8.9×10^{-3} μg g^{-1}, respectively. Steroid estrogen recoveries ranged from 46.9 to 114.3% for the aqueous and particulate phases.

2.4.2. Nonylphenolics

Similarly, all samples for nonylphenolic analysis were separated into aqueous and particulate phases and analysed individually. Analysis utilised a single stage solid phase extraction and quantitation by UPLC-MS/MS which has been detailed elsewhere (Petrie et al., 2013b). The triple quadrupole monitored two MRMs (quantifier and qualifier) for each chemical. Nonylphenolic MDLs ranged from 1.4×10^{-3} to 4.5×10^{-2} μg l^{-1} for aqueous phases and from 1.4×10^{-3} to 3.9×10^{-2} μg g^{-1} for particulate phases. Recoveries for all nonylphenolic chemicals from the aqueous and particulate phases of wastewaters ranged from 40.3 to 102.1% during the studies.

2.4.3. Metals

Sample preparation for metals analysis was similar to that reported by Santos et al. (2010). Briefly, samples were filtered (0.45 μm) to obtain the aqueous phase. Analysis of the unfiltered sample was also performed to determine total concentration. Particulate concentration was then calculated from the difference of the total and aqueous concentrations. Metals analysis was also undertaken on receiving crude sewage to monitor removal over primary sedimentation due to their relatively high affinity to suspended particulate matter. Samples were analysed by an ELAN 9000 inductively coupled

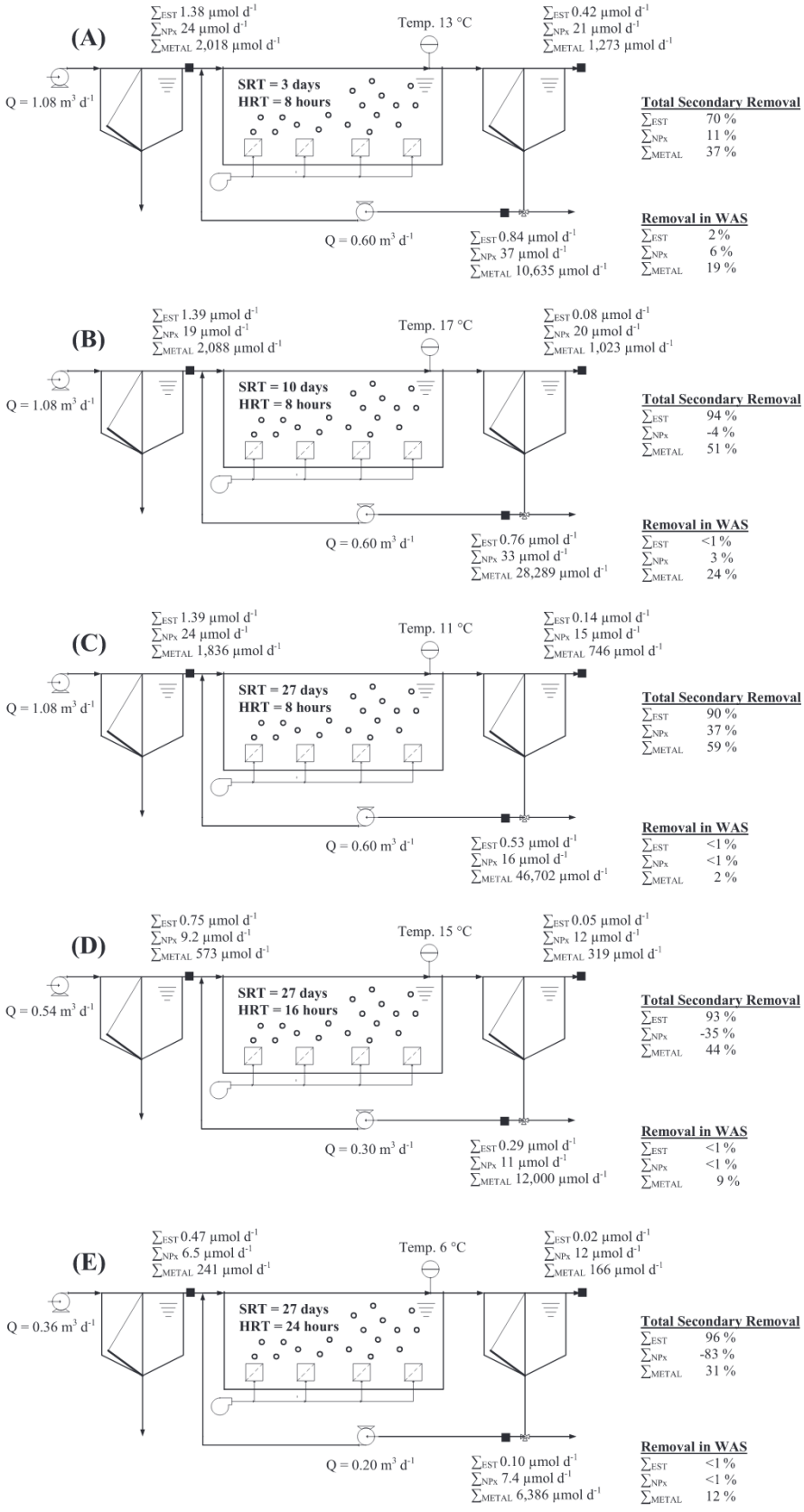

Fig. 1 – Mass balance data for steroid estrogens, NPx and metals at varying operational conditions (A, 3 day SRT 8 h HRT; B, 10 day SRT 8 h HRT; C, 27 day SRT 8 h HRT; D, 27 day SRT 16 h HRT; E, 27 day SRT 24 h HRT; ■, sampling points).

Table 1 – Micropollutant quantitative information reported as mean for SRT and HRT pilot plant studies ($n = 7$).

Chemical	Crude sewage ($\mu g\ l^{-1}$)			SRT (d)	HRT (h)	Settled sewage ($\mu g\ l^{-1}$)			Primary removal[a] (%)	RAS ($\mu g\ l^{-1}$)			Final effluent ($\mu g\ l^{-1}$)			Secondary removal[b] (%)		
	Aq.	Part.	Total			Aq.	Part.	Total		Aq.	Part.	Total	Aq.	Part.	Total	Aq.	Part.	Total
Zn	11.2	80.1	91.3	3	8	11.6	61.5	73.1	14[c]	15.4	553	568	16.5	29.8	46.3	−42	52	38
				10	8	25.1	63.4	88.5		69.5	1.50×10^3	1.57×10^3	35.2	8.90	44.1	−40	86	50
				27	8	16.4	58.7	75.1		39.9	2.82×10^3	2.86×10^3	31.4	6.60	38.0	−91	89	49
				27	16	8.27	32.9	41.2	55	25.6	1.04×10^3	1.07×10^3	22.6	5.10	27.7	−173	85	33
				27	24	9.03	16.8	25.8	72	20.4	921	941	19.2	2.90	22.1	−113	83	14
Cu	1.50	44.3	45.8	3	8	2.90	42.7	45.6	16[c]	2.37	546	548	3.90	24.4	28.3	−34	43	38
				10	8	2.69	33.5	36.2		5.50	1.39×10^3	1.39×10^3	4.40	12.5	16.9	−64	63	53
				27	8	1.72	32.3	34.0		3.82	2.13×10^3	2.13×10^3	2.40	3.80	6.20	−40	88	82
				27	16	1.20	25.8	27.0	41	4.41	1.42×10^3	1.42×10^3	4.23	6.07	10.3	−253	76	62
				27	24	1.45	15.7	17.1	63	4.11	1.10×10^3	1.10×10^3	4.21	3.28	7.48	−190	79	56
Pb	0.673	2.97	3.64	3	8	0.750	1.39	2.14	70[c]	0.500	24.0	24.5	0.600	0.880	1.48	15	51	31
				10	8	0.290	0.720	1.01		0.500	78.9	79.4	0.400	0.317	0.717	−110	56	29
				27	8	<0.116	0.111	0.227		0.150	60.4	60.6	<0.116	-	<0.116	–	–	>49
				27	16	0.260	0.553	0.813	78	0.470	46.8	47.3	0.430	-	0.426	−65	23	48
				27	24	0.550	0.220	0.770	78	0.620	10.2	10.8	0.500	5.60×10^{-2}	0.556	9	75	28
Cd	–	–	–	3	8	$<9.56 \times 10^{-2}$	0.107	0.203	–	$<9.56 \times 10^{-2}$	1.84	1.94	0.110	6.00×10^{-3}	0.116	−15	99	43
				10	8	$<9.56 \times 10^{-2}$	6.54×10^{-2}	0.161		$<9.56 \times 10^{-2}$	6.15	6.25	$<9.56 \times 10^{-2}$	-	$<9.56 \times 10^{-2}$	-	–	>41
				27	8	$<9.56 \times 10^{-2}$	8.74×10^{-2}	0.183		$<9.56 \times 10^{-2}$	3.74	3.84	$<9.56 \times 10^{-2}$	-	$<9.56 \times 10^{-2}$	-	–	>48
				27	16	$<9.56 \times 10^{-2}$	–	$<9.56 \times 10^{-2}$	–	$<9.56 \times 10^{-2}$	2.60	2.70	$<9.56 \times 10^{-2}$	-	$<9.56 \times 10^{-2}$	-	–	–
				27	24	$<9.56 \times 10^{-2}$	–	$<9.56 \times 10^{-2}$	–	$<9.56 \times 10^{-2}$	1.08	1.18	$<9.56 \times 10^{-2}$	-	$<9.56 \times 10^{-2}$	-	–	–
Ni	–	–	–	3	8	0.580	0.740	1.32	–	0.530	15.6	16.1	0.400	0.591	0.991	31	20	25
				10	8	0.130	0.207	0.207		0.300	52.2	52.5	0.140	3.60×10^{-2}	0.176	−8	83	15
				27	8	0.490	0.347	0.837		1.87	44.7	46.6	0.490	0.167	0.657	0	52	22
				27	16	<0.120	–	<0.120	–	<0.120	11.4	11.5	<0.120	-	<0.120	-	–	–
				27	24	<0.120	–	<0.120	–	<0.120	4.20	4.32	<0.120	-	<0.120	-	–	–
E1	–	–	–	3[d]	8	8.91×10^{-2}	2.60×10^{-3}	9.17×10^{-2}	–	0.104	8.60×10^{-2}	0.190	7.40×10^{-2}	5.02×10^{-4}	7.45×10^{-2}	17	81	19
				10[d]	8	9.56×10^{-2}	5.40×10^{-3}	0.101		9.01×10^{-2}	0.104	0.194	6.72×10^{-3}	4.20×10^{-4}	7.14×10^{-3}	93	92	93
				27[d]	8	9.12×10^{-2}	2.80×10^{-3}	9.40×10^{-2}		6.51×10^{-2}	6.50×10^{-2}	0.131	1.46×10^{-2}	1.98×10^{-4}	1.48×10^{-2}	84	93	84
				27	16	0.100	1.98×10^{-3}	0.102	–	8.60×10^{-2}	8.20×10^{-2}	0.168	1.42×10^{-2}	3.98×10^{-4}	1.46×10^{-2}	86	80	86
				27	24	7.17×10^{-2}	2.01×10^{-3}	7.37×10^{-2}	–	3.56×10^{-3}	5.84×10^{-2}	6.20×10^{-2}	4.90×10^{-3}	2.10×10^{-5}	4.92×10^{-3}	93	99	93
E2	–	–	–	3[d]	8	2.58×10^{-2}	4.30×10^{-3}	3.01×10^{-2}	–	3.38×10^{-2}	7.22×10^{-2}	0.106	8.70×10^{-3}	1.01×10^{-3}	9.71×10^{-3}	66	77	68
				10[d]	8	2.23×10^{-2}	6.20×10^{-3}	2.85×10^{-2}		1.30×10^{-2}	7.62×10^{-2}	8.92×10^{-2}	2.21×10^{-3}	7.11×10^{-4}	2.92×10^{-3}	90	89	90
				27[d]	8	2.67×10^{-2}	3.70×10^{-3}	3.04×10^{-2}		2.61×10^{-3}	5.57×10^{-2}	5.83×10^{-2}	2.22×10^{-3}	1.41×10^{-4}	2.36×10^{-3}	92	96	92
				27	16	3.32×10^{-2}	7.49×10^{-3}	4.07×10^{-2}	–	1.11×10^{-2}	2.96×10^{-2}	4.07×10^{-2}	3.18×10^{-3}	1.48×10^{-4}	3.33×10^{-3}	90	98	92
				27	24	3.04×10^{-2}	1.13×10^{-2}	4.17×10^{-2}	–	2.10×10^{-3}	3.41×10^{-2}	3.62×10^{-2}	2.12×10^{-3}	4.99×10^{-5}	2.17×10^{-3}	93	99	95
E3	–	–	–	3[d]	8	0.219	1.12×10^{-3}	0.220	–	1.49×10^{-2}	2.81×10^{-2}	4.30×10^{-2}	1.66×10^{-2}	3.96×10^{-4}	1.70×10^{-2}	92	65	92
				10[d]	8	0.212	2.04×10^{-3}	0.214		8.60×10^{-4}	2.11×10^{-2}	2.20×10^{-2}	6.14×10^{-3}	6.09×10^{-4}	6.75×10^{-3}	97	70	97
				27[d]	8	0.203	1.98×10^{-3}	0.205		1.65×10^{-2}	8.40×10^{-3}	2.29×10^{-2}	5.83×10^{-3}	3.92×10^{-4}	6.22×10^{-3}	97	80	97
				27	16	0.228	6.11×10^{-3}	0.234	–	9.39×10^{-4}	1.66×10^{-2}	1.75×10^{-2}	3.83×10^{-3}	8.23×10^{-5}	3.91×10^{-3}	98	99	98
				27	24	0.232	1.02×10^{-3}	0.233	–	2.70×10^{-3}	1.93×10^{-2}	2.20×10^{-2}	4.08×10^{-3}	5.11×10^{-5}	4.13×10^{-3}	98	95	98

EE2	–	–	–	3[d]	8	6.10×10^{-4}	1.17×10^{-3}	1.78×10^{-3}	–	3.00×10^{-4}	3.26×10^{-2}	3.29×10^{-2}	8.65×10^{-4}	3.95×10^{-4}	1.26×10^{-3}	−40	66	30
				10[d]	8	5.10×10^{-4}	3.96×10^{-4}	9.06×10^{-4}		4.30×10^{-4}	1.99×10^{-2}	2.03×10^{-2}	6.30×10^{-4}	1.70×10^{-5}	6.47×10^{-4}	−24	96	29
				27[d]	8	1.04×10^{-3}	4.51×10^{-4}	1.49×10^{-3}		3.40×10^{-4}	1.45×10^{-2}	1.48×10^{-2}	7.00×10^{-4}	1.80×10^{-4}	8.80×10^{-4}	33	60	41
				27	16	3.99×10^{-4}	1.73×10^{-4}	5.72×10^{-4}	–	6.00×10^{-5}	1.12×10^{-2}	1.13×10^{-2}	1.64×10^{-4}	9.20×10^{-5}	2.56×10^{-4}	59	47	55
				27	24	2.68×10^{-4}	1.30×10^{-4}	3.98×10^{-4}	–	8.00×10^{-5}	1.32×10^{-2}	1.33×10^{-2}	1.22×10^{-4}	1.80×10^{-5}	1.40×10^{-4}	55	86	65
$E_{1-3}S$	–	–	–	3	8	2.21×10^{-2}	3.01×10^{-4}	2.24×10^{-2}	–	7.60×10^{-3}	1.07×10^{-2}	1.83×10^{-2}	4.63×10^{-3}	9.06×10^{-5}	4.72×10^{-3}	79	70	79
				10	8	2.36×10^{-2}	1.95×10^{-4}	2.38×10^{-2}		8.07×10^{-3}	2.05×10^{-2}	2.86×10^{-2}	3.42×10^{-3}	2.96×10^{-5}	3.45×10^{-3}	86	85	85
				27	8	4.08×10^{-2}	1.99×10^{-4}	4.10×10^{-2}		1.65×10^{-2}	6.50×10^{-3}	2.33×10^{-2}	1.45×10^{-2}	1.96×10^{-4}	1.47×10^{-2}	64	2	64
				27	16	1.96×10^{-2}	1.02×10^{-4}	1.97×10^{-2}	–	2.23×10^{-2}	5.31×10^{-3}	2.76×10^{-2}	5.61×10^{-3}	2.11×10^{-5}	5.63×10^{-3}	71	79	71
				27	24	2.40×10^{-2}	2.04×10^{-4}	2.42×10^{-2}	–	4.90×10^{-3}	6.21×10^{-3}	1.11×10^{-2}	4.36×10^{-3}	2.11×10^{-5}	4.38×10^{-3}	82	90	82
NP	–	–	–	3	8	1.02	0.560	1.58	–	0.653	1.84	2.50	1.29	0.444	1.74	−26	21	−10
				10	8	0.479	0.583	1.06		0.641	2.68	3.32	0.876	0.503	1.38	−83	14	−30
				27	8	1.13	0.534	1.66		0.669	0.920	1.59	1.40	0.360	1.76	−23	33	−6
				27	16	0.225	2.26×10^{-2}	0.248	–	$<9.00 \times 10^{-3}$	1.16	1.17	$<9.00 \times 10^{-3}$	6.42×10^{-2}	7.32×10^{-2}	>96	−184	70
				27	24	0.533	9.56×10^{-2}	0.629	–	0.247	0.794	1.04	0.231	3.37×10^{-3}	0.234	57	96	63
$NP_{1-3}EC$	–	–	–	3	8	0.144	1.11×10^{-2}	0.155	–	1.44	7.09	8.54	1.30	2.30×10^{-2}	1.32	−803	−107	−751
				10	8	0.144	2.20×10^{-2}	0.166		1.42	4.98	6.41	2.43	1.10×10^{-2}	2.44	−1588	49	−1370
				27	8	0.168	1.01×10^{-2}	0.178		0.362	3.04×10^{-3}	0.365	0.554	1.16×10^{-3}	0.555	−230	81	−212
				27	16	3.11	1.04×10^{-2}	3.12	–	8.55	0.389	8.94	6.92	1.43×10^{-3}	6.92	−123	86	−122
				27	24	2.52	6.00×10^{-3}	2.53	–	8.71	0.445	9.16	10.1	2.10×10^{-3}	10.1	−301	65	−297
$NP_{1-3}EO$	–	–	–	3	8	1.60	0.929	2.53	–	1.70	3.78	5.49	1.67	0.124	1.80	−4	87	30
				10	8	1.25	0.909	2.16		0.634	4.39	5.02	0.915	0.224	1.14	27	75	48
				27	8	1.22	0.879	2.10		0.463	4.48	4.94	0.729	0.303	1.03	40	66	52
				27	16	0.296	2.11×10^{-3}	0.298	–	2.20×10^{-2}	1.09×10^{-4}	2.20×10^{-2}	1.94×10^{-2}	6.99×10^{-4}	2.01×10^{-2}	93	67	93
				27	24	0.395	0.153	0.548	–	0.132	0.280	0.411	1.98×10^{-2}	5.10×10^{-4}	2.03×10^{-2}	95	99	96
$NP_{4-12}EO$	–	–	–	3	8	2.24	0.504	2.74	–	0.165	2.17	2.33	0.408	8.62×10^{-2}	0.494	82	83	82
				10	8	1.95	0.736	2.68		0.157	1.61	1.77	0.221	4.79×10^{-2}	0.269	89	93	89
				27	8	3.18	0.369	3.55		0.103	0.356	0.460	0.195	7.28×10^{-2}	0.268	94	79	92
				27	16	2.65	0.295	2.95	–	3.92×10^{-2}	1.01	1.05	0.105	0.123	0.229	96	58	93
				27	24	2.56	0.510	3.07	–	0.156	0.932	1.09	0.223	2.61×10^{-2}	0.245	92	95	92

Key: Aq., aqueous; Part., particulate; SRT, solids retention time; HRT, hydraulic retention time; RAS, return activated sludge; –, not determined.

[a] Primary removal (%) = crude sewage − settled sewage/crude sewage 100%.

[b] Secondary removal (%) = settled sewage − final effluent/settled sewage 100%

[c] Mean primary removal from three sampling campaigns.

[d] Petrie et al., 2014.

plasma-mass spectrometer (Perkin Elmer, Beaconsfield, UK). Metal MDLs ranged from 6.6 × 10^{-2} to 0.5 μg l^{-1}. Total and aqueous metal recoveries ranged from 73.1 to 106.9%. Full descriptions of all micropollutant methods are available in Supplementary Material.

3. Results and discussion

3.1. Impact of ASP operation on micropollutant removal

3.1.1. Steroid estrogens

The SRT range studied broadly encompassed typical full-scale operations (Joss et al., 2004; Clara et al., 2005; Johnson et al., 2005; Koh et al., 2009; McAdam et al., 2010, 2011). Process performance at each operating condition studied was ascertained by calculating micropollutant removal according to:

$$\text{Secondary removal}(\%) = \frac{SS - FE}{SS} \cdot 100\% \quad (1)$$

Here SS is the concentration of a given micropollutant in settled sewage and FE is the concentration in final effluent. Removal of total estrogens ($\sum_{EST}$, i.e., sum of E1, E2, E3 and EE2 accounting for aqueous and particulate concentrations) at the 3 day SRT condition (8 h HRT) was 70 ± 7% (Fig. 1). Increased SRT (i.e., 10 and 27 days) achieved augmented removals. However, no improvement in $\sum_{EST}$ removal was observed when SRT was increased from 10 to 27 days. Removal of $\sum_{EST}$ was 94 ± 1% and 90 ± 2% respectively. Higher $\sum_{EST}$ removals here were driven by improved natural estrogen (E1, E2 and E3) removals (Table 1). Removals of E1, E2 and E3 individually were ≥84% for 10 and 27 day SRTs. These are within the range of removals previously reported for various full-scale nitrifying processes (Joss et al., 2004; Clara et al., 2005; Johnson et al., 2005; Koh et al., 2009; McAdam et al., 2010). On the other hand, no improvement in EE2 removal was observed at the 10 or 27 day SRT. Mean removals were in the range 29–41% (Table 1). A broad range of EE2 removals by activated sludge treatment have been observed in the literature, ranging from 38 to 98% (Joss et al., 2004; Clara et al., 2005; Koh et al., 2009; McAdam et al., 2010), with no clear relationship apparent between process operation and removal. Despite increasing SRT, the quantity of $\sum_{EST}$ within RAS reduced (Fig. 1). This, coupled with low estrogen removal in WAS (Fig. 1) infers the majority of removal during activated sludge treatment is attributed to biodegradation (Andersen et al., 2005; Petrie et al., 2014).

Steady state HRTs of 8, 16 and 24 h were monitored to represent typical full-scale (dry weather flow) operational conditions (Johnson et al., 2005; Koh et al., 2009; McAdam et al., 2010, 2011). A fixed SRT of 27 days was employed as this exhibited best overall removal performance previously. Removals of $\sum_{EST}$'s were 90 ± 2%, 93 ± 1% and 96 ± 2% for 8, 16 and 24 h HRTs, respectively (Fig. 1). Individually, each estrogen (including EE2) showed improving removal with longer HRT. At 24 h HRT, removals of the natural estrogens E1, E2 and E3 were all ≥93% (Table 1). Most notably EE2 exhibited removals of 65 ± 19% at the 24 h HRT condition (Table 1). Lengthening HRT of the secondary aerobic treatment stage saw a concomitant decrease in food to microorganism (F: M) ratio. Ratios were 0.25 ± 0.03, 0.13 ± 0.02 and 0.05 ± 0.01 gBOD gVSS d^{-1} for 8, 16 and 24 h HRTs, respectively. Interestingly, reducing F:M ratio correlated well (r^2 0.91) with increased secondary removal of $\sum_{EST}$ (Fig. 2). A lower F: M ratio is suggestive of a substrate limitation which may lead to the biodegradation of less-favoured carbon substrates (e.g., steroid estrogens). Coupled with increased contact time for biodegradation, this may explain the observed improvement in biodegradation at longer HRTs (Fig. 1).

3.1.2. Nonylphenolics

Activated sludge treatment initiated NPEO chain shortening with removals of $NP_{4-12}EO$ ≥ 79% for all pilot plant studies (Table 1). However, removal of total nonylphenolic chemicals ($\sum_{NPx}$, sum of NP, $NP_{1-12}EO$ and $NP_{1-3}EC$ accounting for aqueous and particulate concentrations) during studies investigating SRT was relatively low. At 3 and 10 day SRTs removal of $\sum_{NPx}$ was negligible; 6% and −4%, respectively (Fig. 1). Reported removals of total NPx load in the literature are typically higher (Koh et al., 2009; McAdam et al., 2011) and removals up to 95% have previously been observed for a full-scale ASP operated at an SRT of 13 days (Koh et al., 2009). This is because these studies report load as mass (i.e., ng l^{-1}) instead of moles which can overestimate removal for this chemical type (Petrie et al., 2013b). At 27 days SRT removal of 37 ± 5% was achieved (Fig. 1). This suggests an improvement across the NPx removal pathway by assimilation and oxidation of breakdown intermediates. Higher $\sum_{NPx}$ removal was driven by an improved removal of $NP_{1-3}ECs$ (−212 ± 98%) (Table 1). The specific difficulty associated with this family of

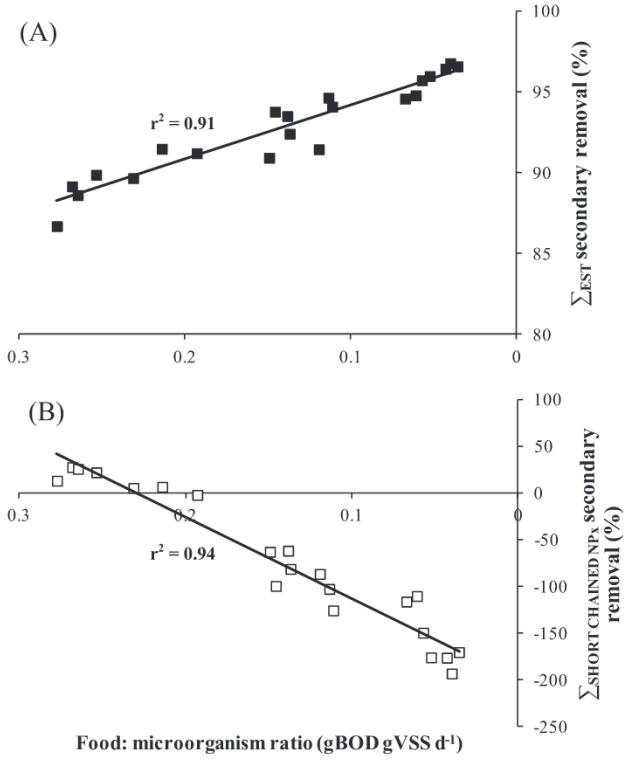

Fig. 2 – Significance of the food: microorganism ratio to secondary removal of $\sum_{EST}$'s (A, ■) and short chained NPx (NP + $NP_{1-3}EO$ + $NP_{1-3}EC$) (B, □) in HRT studies.

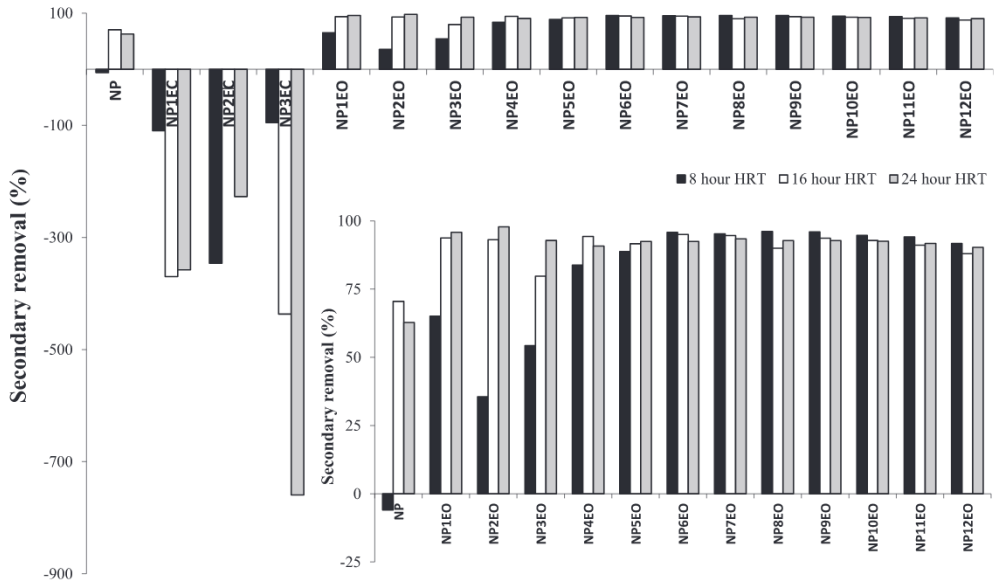

Fig. 3 – Secondary removal of individual nonylphenolic chemicals at varying HRT. Inset, individual removals of NP and $NP_{1-12}EO$.

chemicals is the complexity of biotransformation reactions that this family of chemicals exhibit prior to NP biodegradation. To demonstrate, a number of structural changes (e.g., ethoxylate shortening, oxidations etc) are undertaken before cleavage of the NP ring takes place (Warhurst, 1994; Petrie et al., 2013b). The proposed biotransformation pathway of NPEO's is available in Supplementary Material (Figure S1). In this study, removals of the daughter chemical NP ranged from −6% to −30% over the three SRTs studied (Table 1). As an independent chemical under controlled conditions, NP is known to be susceptible to biodegradation (Tanghe et al., 1998; Stasinakis et al., 2008), even at low SRT (Stasinakis et al., 2010). This suggests its production during activated sludge treatment by precursor biotransformation here was sufficient to compensate for its removal (i.e., net removal is zero).

Lengthening HRT resulted in an apparent increase in $\sum_{NPx}$ molar concentration with removals of 37 ± 5%, −35 ± 18% and −83 ± 12% observed for 8, 16 and 24 h, respectively (Fig. 1). This illustrates that all chemicals within the NPx chemical family are not encompassed by current analytical methods. For example, longer chained NPEOs (Petrie et al., 2013b) and NPECs (Komori et al., 2006), and carboxylated carboxylates (i.e., NP molecule containing two carboxylic acid functional groups) (Jonkers et al., 2001) can be present. Without analytical reference standards available for their quantitation and inclusion in the nonylphenolic mass balance, knowledge of their fate during treatment and resultant impact to NP removal remains limited. A negative correlation (r^2 0.94) was observed between reduced F:M ratio and short chained NPx removal (i.e., NP + $NP_{1-3}EO$ + $NP_{1-3}EC$) (Fig. 2). This was mainly attributed to increased concentrations of $NP_{1-3}EC$ observed in final effluents (Table 1). Nevertheless, this counter-intuitively resulted in the greatest removals of NP. Positive removals of 70 ± 16% and 63 ± 15% were observed at 16 and 24 h HRTs respectively (Fig. 3). Removals of >50% have previously been reported in the literature for NP (Nakada et al., 2006; Qiang et al., 2013), but these have not been supported with parent compound analysis which is needed to better understand pathways of biotransformation. The high removal of all NPEOs observed at 16 and 24 h HRTs (Table 1) indicates that ethoxylate biotransformation was the dominant pathway for the production of NP during ASP treatment. Therefore longer HRT was advantageous as this initiated improved biotransformation across the NPx removal pathway resulting in notable NP removal. It should be noted that the vapour pressure (4.39×10^{-5} kPa) and Henry's law constant (4.30×10^{-6} atm m^3mol^{-1}) (Table S1) of NP indicates a semi-volatile nature (Soares et al., 2008). Therefore increased removal by volatilisation at longer HRTs cannot be discounted.

3.1.3. *Metals*

In contrast to the organic micropollutants, total metal ($\sum_{METAL}$, sum of Zn, Cu, Pb, Cd and Ni accounting for aqueous and particulate concentrations) removal increased moderately with each SRT increment. Removals were 34 ± 19%, 51 ± 10% and 59 ± 11% for 3, 10 and 27 day SRTs respectively (Fig. 1). This was mainly attributed to Cu and Zn as they constituted the majority of metals concentration found in receiving sewage (Table 1). Specifically, Cu removals were improved by increasing SRT and were 38 ± 15%, 53 ± 9% and 82 ± 4%. In contrast, Ni removals were low and in the range; 15–25%. The difficulty of removing Ni during biological wastewater treatment is well established (Ziolko et al., 2011). Partitioning within the biomass matrix was confirmed as the main removal pathway for metals as increased concentrations were observed in the particulate phase of RAS at each SRT increase (Fig. 1, Table 1). The moderate improvement (8%) in $\sum_{METAL}$ removal between the 10 and 27 SRTs coincided with similar MLVSS concentrations (Fig. 1, Table S2). This suggests enhanced metal partitioning within the biomass matrix, likely to be induced by a physiological change to the biomass

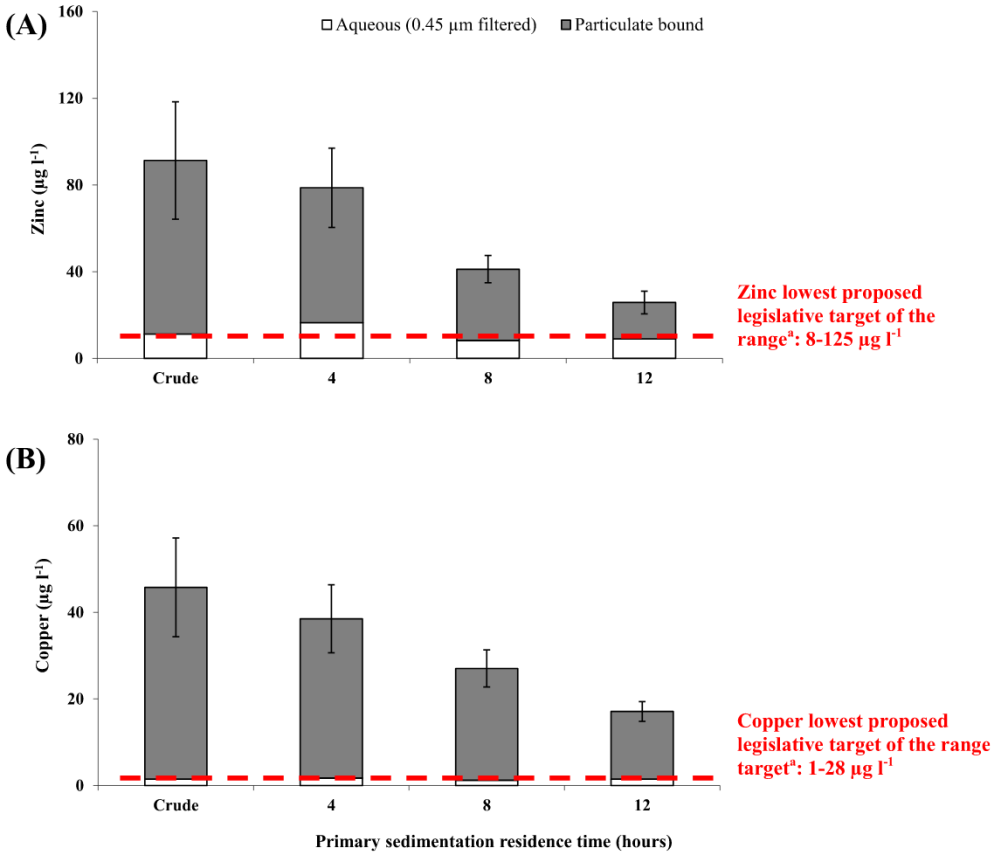

Fig. 4 – Effect of primary sedimentation tank residence time on Zn (A) and Cu (B) concentration in settled sewage. [a]EQS is applied to the aqueous concentration (i.e., pre-filtered) and is dependent on water hardness (European Commission, 2008). Note: error bars represent the standard deviation of the total concentration.

composition. For example, Laurent et al. (2009) reported that smaller floc size was critical for increased Cd sorption. Activated sludge (of similar dry weight) comprised of smaller flocs has better availability of binding sites due to increased floc surface area. In this study median floc sizes reduced from 274 to 164 µm between SRTs of 10 and 27 days (Table S2). This may explain improved removal achieved by the 27 day SRT biomass. However, metal concentrations in the aqueous phase of wastewater (to which EQS's are applied) increased during treatment at all SRTs (Table 1). Aqueous removals of Cu and Zn ranged from −34 to −91% at 3, 10 and 27 day SRTs. Increased aqueous metal concentration is characteristic of ASP treatment and aqueous removals of −170% have previously been observed for Zn at an SRT of 8 days (Santos et al., 2010).

Lengthening HRT of the pilot plant system initially resulted in increased removal of $\sum_{METAL}$'s observed during primary sedimentation (prior to secondary treatment). This was a result of a high proportion of metals found in crude sewage being associated with particulates. For example, the percentage of Zn and Cu within the particulate phase (>0.45 µm) of crude wastewater was 88 ± 4% and 97 ± 2%, respectively (Fig. 4). Suspended solids concentration of crude sewage was 366 ± 154 mg l^{-1} and reduced to 123 ± 15, 88 ± 7 and 64 ± 13 mg l^{-1} in settled sewage for primary sedimentation times of 4, 8 and 12 h respectively (Table S3). This corresponded well to metal behaviour. To demonstrate, $\sum_{METAL}$ concentrations in crude sewage were 141 ± 29 µg l^{-1} with the majority of this attributed to Zn (91 ± 27 µg l^{-1}) and Cu (46 ± 11 µg l^{-1}) (Fig. 4). In settled sewage Zn concentrations were 79 ± 18, 41 ± 6 and 26 ± 5 µg l^{-1} corresponding to primary sedimentation residence times of 4, 8 and 12 h (Table 1). Similarly, total Cu concentrations were reduced to 39 ± 8, 27 ± 4 and 17 ± 2 µg l^{-1} in settled sewage. Metal concentrations found in settled sewage were therefore reduced as HRT of the pilot plant was lengthened.

During secondary treatment, removal of $\sum_{METAL}$'s decreased with longer HRT. This is explained by a linear correlation (r^2 0.92) with reduced $\sum_{METAL}$ concentrations in settled sewage (as primary sedimentation residence time increased) (Fig. 5). Secondary treatment at longer HRT also resulted in increased production of aqueous metals (Fig. 5). To illustrate, aqueous metals removals were −88 ± 9% at an 8 h HRT whereas removals at 16 and 24 h HRTs were −176 ± 9% and −128 ± 6% respectively. Individually, Cu exhibited the greatest negative removal. Secondary removals were −40 ± 12% (8 h HRT), −253 ± 53% (16 h HRT) and −190 ± 24% (24 h HRT). It is postulated that longer contact times may facilitate further oxidation of particulate organic matter resulting in the increased solubilisation of metals (Ziolko et al., 2011). Also, products of bacterial metabolism and the production of extracellular polymers sloughed from bacterial

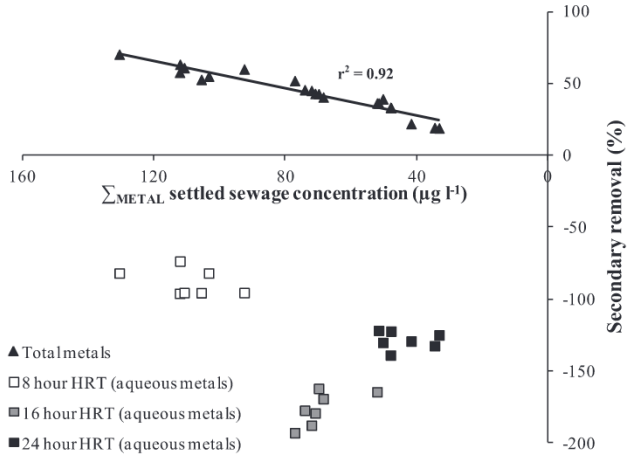

Fig. 5 – Impact of $\sum_{METAL}$ concentration (i.e., sum of aqueous and particulate concentrations of Zn, Cu, Pb, Cd and Ni) in settled sewage to secondary (total and aqueous metals) removal.

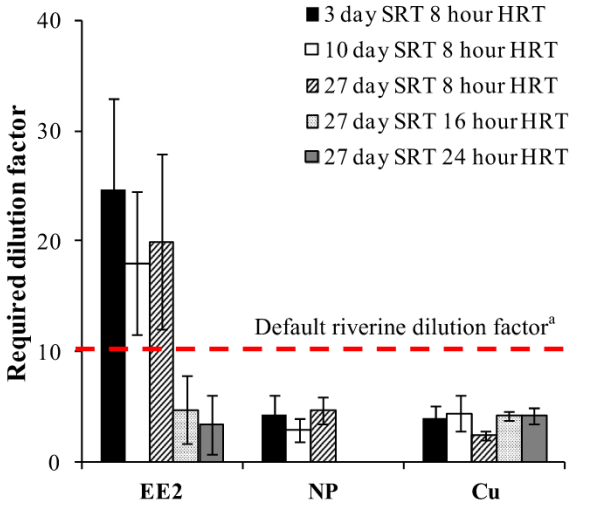

Fig. 6 – Riverine dilution required to ensure consent to EQS and proposed legislative targets for EE2 (proposed legislative target = 3.5×10^{-5} µg l^{-1}, NP (EQS = 0.3 µg l^{-1}) and Cu (proposed legislative target = 1–28 µg l^{-1}) at each ASP operation. [a]Default riverine dilution factor of 10 as described by the European Commission (European Commission, 2003) Note: Dilution factor required for consent is calculated from the aqueous (i.e., pre-filtered) concentration only (European Commission, 2008). Micropollutant concentration already present in dilution water is assumed to be zero. For metals, a calcium carbonate concentration of 0–50 mg l^{-1} is assumed as EQSs are dependent on water hardness.

cells could help retain metals in the bulk solution at longer HRTs (Santos et al., 2010). This increased solubilisation of metals at longer HRT is significant as only the aqueous (i.e., pre-filtered) phase of water matrices is to be considered for EQS compliance (European Commission, 2008).

3.2. *Final effluent quality and the prospect of achieving environmental compliance*

To assess the likelihood of achieving compliance for each micropollutant within the receiving aqueous environment, the level of final effluent dilution required for consent was determined (Fig. 6). This denotes the minimum river dilution requirement to achieve the EQS or proposed legislative target for each ASP final effluent for EE2, NP and Cu (only aqueous concentrations are considered for compliance). These chemicals were selected as they required the most dilution in this study within the micropollutant groups; steroid estrogens, NPx's and metals. Compliance was unequivocally assured for a default dilution factor of 10 (European Commission, 2003) by the 27 day SRT 16 and 24 h HRT operations. Modifying ASP operation had the greatest impact to the dilution requirement for EE2. This was due to EE2 having a very low proposed legislative target of 3.5×10^{-5} µg l^{-1} (European Commission, 2012; Table S1) and it being more responsive to changes in HRT. Dilution of ≥20 times was needed to ensure consent for ASPs where SRT was investigated (Fig. 6). Conversely, at 16 and 24 h HRTs the dilution required was ≤10 due to increased removals of EE2. In comparison, the required dilution for consent for NP was <10 for all ASP final effluents. However, despite NP being the only chemical within the NPx group which has an EQS, it has been successfully demonstrated that the biotransformation of readily degradable NP precursors in the environment (which are not legislated for) could theoretically lead to a breach of EQS downstream of the effluent discharge point (Petrie et al., 2013b). Molar concentrations of cumulative NPx's in final effluents were observed up to 37 ± 6 µmol m^{-3} (1.4 µmol m^{-3} of NP is equivalent to its EQS of 0.3 µg l^{-1}). It is therefore advantageous to operate the ASP at conditions which enhance biotransformation across the NPx family of chemicals (i.e., high SRT and long HRT conditions).

Interestingly, aqueous metal concentrations (to which EQS's are applied) were similar to, or below their proposed legislative targets for consent in crude and settled sewages (Fig. 4). Following aerobic treatment, aqueous concentrations were above these targets for both Cu and Zn. The 27 day SRT 8 h HRT achieved the best final effluent quality with respect to aqueous Cu concentrations. The required dilution factor needed to achieve the proposed legislative target for consent was 2.4 ± 0.4 times (Fig. 6). Despite lower settled sewage concentrations at longer HRTs due to improved removal of particulate bound metals during primary sedimentation, no improvement in the required dilution factor for consent was observed. This is because increased solubilisation of the remaining particulate bound phase was observed. However, the dilution requirement is likely to be greater for a full-scale process operating at similar HRT conditions. This is because primary sedimentation tanks are not designed for the longer residence times observed in this study and therefore, settled sewage metal concentrations are likely to be considerably greater. For example, influent wastewaters of full-scale processes commonly contain Cu and Zn at concentrations >100 µg l^{-1} (Rule et al., 2006; Ziolko et al., 2009; Gardner et al., 2013). Consequently, poorer secondary removal of aqueous

metals at longer HRT will be more apparent in final effluents here. This increase of aqueous metal concentrations at conditions favourable for organic micropollutant removal demonstrates the difficulty of optimising ASP operation for simultaneous removal of estrogens, NPx and metals.

3.3. *Options for upgrading existing ASPs for simultaneous micropollutant removal*

The level of micropollutant removal required to reach compliance limits for a given ASP will be highly site specific. This will depend on receiving micropollutant concentrations as well as available dilution at the discharge point. Despite the very different behaviour observed for these micropollutants during ASP treatment, operating conditions can be modified to improve their removal simultaneously. The majority of existing full-scale ASPs are designed to operate at mid-ranged conditions (i.e., *circa* 10 day SRT 8 h HRT) at yearly average flows (Aboobakar et al., 2013). Yet these tend to receive significant variations in sewage flow resulting in a dynamic system with significant variations in SRT and HRT. Therefore improvements in full-scale performance are achievable by better process control to improve process continuity and reduce variations in both SRT and HRT. For example, the use of *in-situ* suspended solids probes and real-time flow measurements could help avoid variability in SRT. It was demonstrated that operation at a higher SRT improved simultaneous micropollutant removal. However, higher SRT operation has traditionally been associated with significantly greater aeration demands. Recently it has been shown that operation at a higher SRT achieves improved oxygen transfer due to smaller activated sludge floc size and better uniformity (Leu et al., 2012). Consequently, aeration requirements are not as great as previously considered.

Achieving a significant improvement to HRT will likely require infrastructure development. Where sufficient space is not available to achieve a desired HRT, continuous use of onsite storm tanks or remote holding tanks could act as a buffer to counter-balance fluctuations in sewage flow, enabling process continuity whilst operation at a longer HRT. Furthermore, lengthening secondary HRT is likely to require improved metals removal during primary treatment as they behaved very differently to organics during biological treatment. Therefore, maximising particulate bound metals removal during primary sedimentation to circumvent their solubilisation during secondary treatment is essential. This is critical for sites which have low dilution ratios or where the receiving concentrations of metals are comparatively greater. This study successfully demonstrated that primary sedimentation tanks operated at longer HRT can effectively enhance metal removal prior to secondary treatment (Fig. 4). However, enhancing suspended solids removal by existing assets may not be achievable without the continual dosing of coagulants. For example, there may not be sufficient space available onsite to increase primary sedimentation tank size to facilitate HRTs similar to those utilised here. As a trade-off, the alternative or complimentary use of micro-screens can yield improved suspended solids removal at a comparatively smaller footprint (Salsnes Filter, 2011).

4. Conclusions

- Prospects of achieving EQS compliance through ASP operation improvements are highly site specific, governed by receiving micropollutant concentrations in crude sewage, available dilution water and quality, and available onsite space for infrastructure development.
- Solids retention times of 10 and 27 days achieved greater $\sum_{EST}$ removal and an increase of SRT from 10 to 27 days achieved greater $\sum_{NPx}$ and $\sum_{METAL}$ removal.
- Longer HRT of the aerobic treatment stage was vital for achieving increased biodegradation of $\sum_{EST}$ (specifically EE2) and suitable precursor biotransformation such that positive NP removals are achieved.
- Enhanced removal of particulates from crude wastewater during primary sedimentation is critical for improved metal removals and avoiding increased concentrations of aqueous metals following secondary treatment at longer HRT.
- This study has identified that the broad range of micropollutant chemistries do result in trade-offs which should be considered for future studies.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2014.05.036.

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Assessing potential modifications to the activated sludge process to improve simultaneous removal of a diverse range of micropollutants

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Supporting information

The supporting information contains detailed information of steroid estrogen, nonylphenolic and metals analysis. It also includes three tables (Table S1, S2 and S3) containing information on the physico-chemical properties of the micropollutants studied, findings from biomass characterisations and quantitative information of sanitary determinand analysis from pilot plant studies and one Figure (Figure S1) showing the proposed breakdown degradation pathway of nonylphenol ethoxylates to nonylphenol.

Steroid estrogen analysis

Aqueous extraction

Settled sewage and final effluent samples (>1 litre) were filtered under vacuum through 1.2 µm GF/C filters (VWR, Lutterworth, UK) prior to solid phase extraction (SPE). Filter papers with retained solids were then frozen. Waste activated sludge (1 litre) was centrifuged at 1,500 x *g* and filtered to remove solids. These solids were also frozen – see particulate extraction. The aqueous fraction (1 litre per sample) was loaded onto 500 mg:6 cc tC18 solid phase extraction (SPE) cartridges (Waters, Elstree, UK) preconditioned with 5 ml methanol (MeOH) then 5 ml ultra-pure (UP) water. Cartridges changes were made after 500 ml to avoid cartridge saturation. The flow rate was controlled at 5 ml min^{-1} using a vacuum manifold. The cartridge was then washed with 5 ml UP H_2O and dried for 60 minutes under vacuum. Analytes were eluted using 10 ml MeOH followed by 10 ml dichloromethane (DCM). Extracts were concentrated to 1 ml by rotary evaporation (Heidolph Instruments, Schwabach, Germany) then to complete dryness under a gentle stream of nitrogen gas. The dried sample was reconstituted in 0.2 ml DCM: MeOH (90: 10). The sample was then subject to clean up by gel permeation chromatography (GPC) by injection onto a 5 µm, 300 mm x 7.5 mm gel permeation size exclusion column (Varian, Oxford, UK) under isocratic conditions (DCM: MeOH, 90: 10 v/v) at a constant flow rate of 1 ml min^{-1} and the fraction between 5.5 minutes and 11.5 minutes collected. The 6.0 ml fraction was evaporated to approximately 0.2 ml under a nitrogen stream then reconstituted to 2 ml with hexane. This was loaded onto a preconditioned (2 ml hexane) 500 mg:6 cc NH_2 SPE cartridge (Varian, Oxford, UK), at a flow rate of 5 ml min^{-1}. The cartridge was then washed using 4 ml ethylacetate (EtOAc): hexane (10: 90). Non polar estrogens (E1, E2 and EE2) were eluted using 6 ml EtOAc. The more polar estrogens (E3 and E1-3S) were then eluted separately using 6 ml of 3 % ammonium hydroxide (NH_4OH) in MeOH. Separate eluants were blown to dryness under a gentle stream of nitrogen gas, then reconstituted with 0.2 ml UP H_2O: MeOH (80:20). Prior to extraction the aqueous phase samples were spiked with 15 ng l^{-1} deuterated estrogens. To ensure

analytical reliability; both low (2 ng l^{-1}) and high (15 ng l^{-1}) spikes of additional mixed estrogens were used to determine recoveries.

Particulate extraction

Frozen settled sewage, final effluent particulate samples contained on filter papers and sludge samples (0.1-0.2 g) were freeze-dried and extracted using 10 ml EtOAc whilst being mechanically agitated using a multi-reax system (Heidolph Instruments, Schwabach, Germany) in a 25 ml Teflon tube for 60 minutes. This was followed by centrifugation at 1500 x *g* for 10 minutes. The extraction was repeated twice then the combined supernatants were evaporated to approximately 0.2 ml and reconstituted to 2 ml with hexane. The sample was then subjected to SPE by passing through a 500 mg:3 ml silica cartridge (Waters Ltd., Watford, UK) preconditioned with 2 ml hexane at a constant flow rate of 5 ml min^{-1}. Analytes were then eluted using 3 ml EtOAc followed by 2 ml MeOH ensuring the cartridge media remained saturated. Extracts were then evaporated to dryness prior to reconstitution in 0.2 ml DCM: MeOH (90: 10). This was then subject to the same clean up procedure as the aqueous samples by GPC and normal phase SPE. To ensure analytical reliability all samples prior to freeze drying were spiked with deuterated (75 ng g^{-1}) estrogens and both low (10 ng g^{-1}) and high spikes (75 ng g^{-1}) when required to determine recoveries.

UPLC-MS/MS analysis

Ultra performance liquid chromatography was performed using a Waters Acquity UPLC (Waters, Manchester, UK). Separations were achieved using an Acquity UPLC BEH C18 column (100 mm x 2.1 mm i.d., 1.7 µm particle size; Waters, Manchester, UK) maintained at 45 ˚C. A gradient separation of two mobile phases was utilised consisting of water containing 0.1 % NH_4OH (A) and methanol containing 0.1 % NH_4OH (B) at a constant flow rate of 0.2 ml min^{-1}. Initial conditions of 80 % A and 20 % B were increased gradually to 80 % B over 6 minutes and maintained for 1 minute. This was then returned to starting conditions for 2 minutes for equilibration. An injection volume of 10 µl was used throughout the

73 investigation. Detection was achieved by a Waters Quattro Premier XE mass spectrometer
74 with a Z-spray ESI interface (Micromass, Watford, UK). This was operated in the negative
75 electrospray ionisation mode utilising multiple reaction monitoring (MRM). Two MRM
76 transitions were monitored for each estrogen. Detection of estrogens was divided into two
77 acquisition periods. Period 1 monitored for E3 and E1-3S and period 2 for those later eluting,
78 less polar estrogens E1, E2 and EE2. The parameters for detection by the mass spectrometer
79 were as follows: capillary voltage, 3.20 kV; multiplier voltage, 650 V; desolvation gas flow,
80 1000 l h^{-1}; cone, -55 V; RF lens, 0.2 V; cone gas flow, 49 l h^{-1}; desolvation temperature, 350
81 °C and source temperature, 120 °C.
82
83

84 ***Nonylphenolic analysis***

85 **Aqueous extraction**

86 Settled sewage (100 ml) and final effluents (250 ml) were filtered through 1.2 µm GF/C
87 filters (VWR, Lutterworth, UK) prior to SPE. 250 ml of sludge was centrifuged and filtered
88 prior to extraction. Filter papers containing retained particulates were then frozen prior to
89 extraction separately – see particulate extraction. The aqueous fraction was loaded onto 500
90 mg:3 cc tC18 cartridges (Waters, Elstree, UK) preconditioned with 5 ml MeOH then 5 ml UP
91 water. The sample was loaded at a flow rate of 5 ml min^{-1} using a vacuum manifold. A 5 ml
92 aliquot of UP water was then used to wash the cartridge. The cartridge was then dried for an
93 hour under vacuum prior to elution. Analytes were eluted using 5 ml EtOAc, 5 ml 0.1 %
94 acetic acid in MeOH and then 5 ml DCM. Extracts were then concentrated to approximately
95 1 ml by rotary evaporation (Heidolph Instruments, Schwabach, Germany) to complete
96 dryness under a gentle stream of nitrogen gas. Once dried the extract was reconstituted in
97 0.25 ml of UP H_2O: MeOH (80: 20). This was transferred to an autosampler vial prior to
98 quantification by UPLC-MS/MS. To ensure analytical reliability, prior to extraction; both
99 low (100 ng l^{-1}) and high (1,000 ng l^{-1}) spikes of all compounds were used to determine
100 recoveries.
101
102 **Particulate extraction**
103 Particulate phase analysis was performed by solvent extraction of filter papers containing
104 suspended solids (0.1-0.2 g) from filtering >1 litre of settled sewage or final effluent, or
105 centrifugation and filtration of sludge. These were freeze dried, shredded and extracted using
106 10 ml acetone and 10 ml MeOH by mechanically agitating using a multi-reax system
107 (Heidolph Instruments, Schwabach, Germany) in a 25 ml Teflon tube for 30 minutes. The
108 extracts were centrifuged at 1500 x *g* for 10 minutes then decanted off. This process was
109 repeated and the extracts combined and concentrated to 0.2 ml. These were reconstituted to 2
110 ml with hexane and loaded onto a 500 mg:3 ml silica cartridge (Waters Ltd., Watford, UK)
111 preconditioned with 2 ml hexane. Analytes were eluted using 10 ml of 10 % acetic acid in

MeOH then dried and reconstituted in 0.25 ml of UP water: MeOH (80: 20) prior to quantification by UPLC-MS/MS. Before freeze drying, selected samples received either low (100 ng g^{-1}) or high spikes (1,000 ng g^{-1}) of all compounds to determine recoveries.

UPLC-MS/MS analysis

Ultra performance liquid chromatography was performed using a Waters Acquity UPLC (Waters, Manchester, UK). Chromatographic separations were achieved using an Acquity UPLC BEH C18 column (100 mm x 2.1 mm i.d., 1.7 µm particle size; Waters, Manchester, UK) controlled at 30 ˚C. A gradient elution of 0.1 % NH_4OH in UP water (A) and 0.1 % NH_4OH in MeOH (B) at a flow rate of 0.4 ml min^{-1} was used. Initial conditions of 20 % B were maintained for 4 minutes prior to gradually increasing to 80 % over 5 minutes. This was maintained for 12 minutes then returned to starting conditions over 3 minutes. The total run time was 26 minutes and an injection volume of 10 µl was used. Detection was achieved using a Waters Quattro Premier XE mass spectrometer with a Z-spray ESI interface (Micromass, Watford, UK). This was operated in the negative and positive electrospray ionisation mode utilising multiple reaction monitoring (MRM). The parameters for detection by the mass spectrometer were as follows: capillary voltage, 3.20 kV (positive mode) and -2.3 kV (negative mode); extractor lens; 3.0 V, multiplier voltage, 650 V; desolvation gas flow, 1000 l h^{-1}; RF lens, 0.5 V (positive mode) and 1.0 V (negative) mode; cone gas flow, 50 l h^{-1}; desolvation temperature, 350 °C and source temperature, 120 °C.

134 ***Metals analysis***

135 **Glassware**

136 Glass and plastic ware used for collection, storage, filtering and digestion of samples was
137 soaked for a minimum of 24 hours in an aqueous 2.5 % v/v Decon 90 solution (Fisher
138 Scientific, Loughborough, UK) and then rinsed with UP water three times. Subsequently they
139 were soaked for a minimum of 24 hours in 2.5 % v/v OPTIMA trace metal grade nitric acid.
140 Glass and plastic ware was then rinsed a minimum of five times with UP water before being
141 air dried.

142

143 **Total metals preparation**

144 Total metal analysis was achieved by acid digestion. Samples for total metal analysis should
145 contain a maximum of 100 mg of solids; if samples contained more than 100 mg of solids,
146 dilution with acidified ultrapure water was used to reduce the solids content to ≤100 mg
147 (Santos *et al*., 2010). Samples (30 mL) were placed in digestion tubes and acidified with 1.5
148 mL OPTIMA trace metal grade nitric acid. The samples were digested using a MARSXpress
149 microwave digester (CEM Microwave Technology, Buckingham, UK) using sample
150 dissolution EPA method 3015 (USEPA, 2007). The samples were then transferred to 10 mL
151 centrifuge tubes for analysis and spiked with the internal standard rhodium to achieve a
152 concentration of 50 µg l^{-1}. All samples were analysed in duplicate and selected samples were
153 spiked at low (20 µg l^{-1}) and high (100 µg l^{-1}) concentrations to determine recoveries.

154

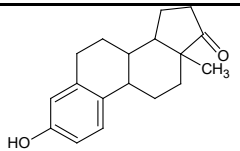
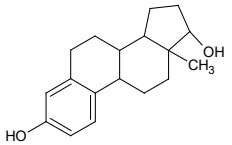
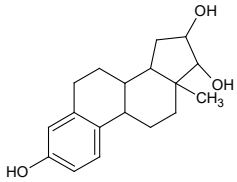
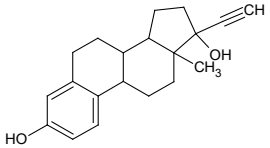
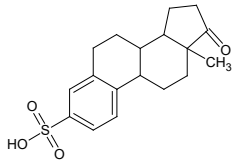
155 **Aqueous metals preparation**

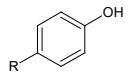
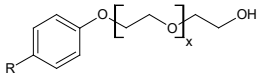
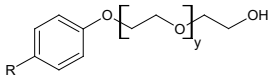
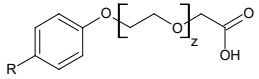
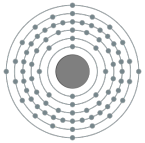
156 For aqueous metal analysis, each sample was vacuum-filtered through a Millipore all-glass
157 three piece vacuum filtering set (Millipore, Cambridge, UK) using a 0.45 µm pore size
158 cellulose nitrate membrane filter (Anachem Ltd, Luton, Bedfordshire, UK). A 10 mL aliquot
159 of the sample filtrate was then placed in a 10 mL centrifuge tube and acidified with 0.75 ml
160 OPTIMA trace metal grade nitric acid and internal standard added. All samples were then
161 refrigerated at 4 °C prior to ICP-MS analysis.

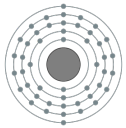
ICP-MS analysis

The RF power was 1 kW with a nebuliser flow rate: 0.77 l min^{-1}. Plasma, auxiliary gas and sheath gas flow rates were all automatically controlled. The sample cone had a 1.1 mm diameter orifice and the skimmer cone with a 0.9 mm diameter orifice. The dwell time was 50ms/AMU and scan mode was peak hopping. The samples were determined in "dual detector mode" and "steady state mode" to introduce the sample continuously.

Table S1. Physicochemical properties of micropollutants found in wastewaters (EPI Suite, 2012)

Chemical of interest	Organic chemical structure/metal electron configuration	EQS/proposed legislative target (µg l^{-1})	Molecular weight (g mol^{-1})	Water solubility (mg l^{-1})	pKa	Vapour pressure (kPa)	Henry's law constant (atm m^3 mol^{-1})	Density (g cm^{-3})	Log K_{ow}	Log K_{oc}
Estrone (E1)		3.0 x 10^{-3}[a]	270.37	30.0	10.50[c]	3.00 x 10^{-8}	3.80 x 10^{-10}	-	3.13-3.43	3.02-4.38
17β-estradiol (E2)		4.0 x 10^{-4}[b]	272.39	3.6	10.71[c]	3.00 x 10^{-8}	3.64 x 10^{-11}	-	3.94-4.01	2.90-4.01
Estriol (E3)		-	288.38	441.0	-	9.00 x 10^{-13}	1.33 x 10^{-12}	-	2.45-2.81	1.62-3.08
17α-ethinylestradiol (EE2)		3.5 x 10^{-5}[b]	296.40	11.3	10.40[c]	6.00 x 10^{-9}	7.94 x 10^{-12}	-	3.67-4.15	2.71-4.65
Estrone 1-3sulfate (E1-3S)		-	350.43	960.0	-	1.97 x 10^{-11}	2.04 x 10^{-12}	-	0.95	1.81

Nonylphenol (NP)		0.3[d]	220.35	7.6	10.28[e]	4.39×10^{-5}	4.30×10^{-6}	0.95[e]	5.77	4.28
Nonylphenol mono, di and tri-ethoxylate ($NP_{1-3}EO$)		-	264.41-352.52	0.5-1.8	-	2.38×10^{-8}-5.24×10^{-11}	1.25×10^{-6}-5.73×10^{-12}	-	5.03-5.58	4.28
Nonylphenol polyethoxylates ($NP_{4-12}EO$)		-	396.57-749.00	3.3-248	-	3.04×10^{-12}-5.43×10^{-20}	1.23×10^{-14}-5.35×10^{-36}	-	2.56-4.75	1.35-3.17
Nonylphenol carboxylates ($NP_{1-3}EC$)		-	278.39-366.50	0.3-1.0	-	1.31×10^{-6}-1.80×10^{-10}	1.79×10^{-7}-4.31×10^{-11}	-	5.26-5.80	2.94-3.42
Zinc (Zn)		8-125[f]	65.39	3.4×10^{5}	-	-	-	7.15[g]	-	-
Copper (Cu)		1-28[f]	63.55	4.2×10^{5}	-	-	-	8.90[g]	-	-
Lead (Pb)		1.2[d]	207.20	9.6×10^{3}	-	-	-	11.34[g]	-	-

Cadmium (Cd)		0.08-0.25[d]	112.41	1.2×10^5	-	-	-	8.70[g]	-	-
Nickel (Ni)		4.0[d]	58.69	4.2×10^5	-	-	-	8.90[g]	-	-

[a]Environment Agency of England and Wales, 2002 [b]European Commission, 2012 [c]Liu *et al*., 2009 [d]European Commission, 2008 [e]Soares *et al*., 2008 [f]Ziolko *et al*., 2011 [g]Lenntech, 2012
Key: EQS, environmental quality standard; pKa, acid dissociation constant; Log K_{ow}, octanol-water coefficient; Log K_{oc}, organic carbon-water coefficient

Table S2. Physiochemical properties of activated sludge, and extracted soluble micro-products and extracellular polymeric substances of varying SRT and HRT

Physicochemical property	SRT (days)	HRT (hours)	Activated sludge	Soluble micro-products	Extracellular polymeric substance
Protein (mg g^{-1} VSS)	3	8[a]	-	41	202
	10	8[a]	-	49	197
	27	8[a]	-	17	158
	27	16	-	13	133
	27	24	-	12	154
Carbohydrate (mg g^{-1} VSS)	3	8[a]	-	21	29
	10	8[a]	-	24	46
	27	8[a]	-	13	43
	27	16	-	10	22
	27	24	-	5	19
COD (mg g^{-1} VSS)	3	8[a]	-	72	438
	10	8[a]	-	30	387
	27	8[a]	-	22	315
	27	16	-	37	333
	27	24	-	38	347
Zeta potential (mV)	3	8[a]	-13	-9	-25
	10	8[a]	-12	-9	-18
	27	8[a]	-11	-6	-18
	27	16	-16	-9	-16
	27	24	-18	-8	-12
MLVSS (mg g^{-1} VSS)	3	8[a]	972	-	-
	10	8[a]	1,301	-	-
	27	8[a]	1,284	-	-
	27	16	1,067	-	-
	27	24	1,125	-	-
d_{50} (μm)	3	8[a]	358	-	-
	10	8[a]	274	-	-
	27	8[a]	164	-	-
	27	16	369	-	-
	27	24	236	-	-

[a]Petrie *et al*., submitted

Key: SRT, solids retention time; HRT, hydraulic retention time; COD, chemical oxygen demand; MLVSS, mixed liquor volatile suspended solids; d_{50}, median floc size

Table S3. Sanitary determinand quantitative information for SRT and HRT pilot plant studies

Sanitary determinand	SRT (days)	HRT (hours)	Settled sewage (mg l^{-1})	Final effluent (mg l^{-1})	Secondary removal (%)
COD	3	8	266	101	62
	10	8	258	52	80
	27	8	291	51	82
	27	16	252	43	83
	27	24	216	42	81
sCOD	3	8	109	42	62
	10	8	76	24	68
	27	8	103	30	71
	27	16	132	29	78
	27	24	103	34	67
BOD	3	8	104	27	74
	10	8	100	12	88
	27	8	105	5	96
	27	16	93	5	94
	27	24	59	2	96
Ammonium	3	8	44	41	6
	10	8	38	<0.5	>99
	27	8	28	<0.5	>99
	27	16	31	<0.5	>99
	27	24	30	<0.5	>99
Nitrate	3	8	<1	<1	-
	10	8	<1	35	<-3,400
	27	8	<1	29	<-2,780
	27	16	<1	34	<-8,425
	27	24	<1	30	<-5,857
Nitrite	3	8	<1	6	<-520
	10	8	<1	3	<-230
	27	8	<1	1	<-20
	27	16	5	<1	>79
	27	24	3	<1	>65
Total nitrogen	3	8	59	53	10
	10	8	52	47	10
	27	8	44	34	23
	27	16	42	38	9
	27	24	47	40	14
Suspended solids	3	8	110	69	37
	10	8	119	29	76
	27	8	139	18	87
	27	16	88	22	74
	27	24	64	17	67

Key: SRT, solids retention time; HRT, hydraulic retention time; COD, chemical oxygen demand; sCOD, soluble chemical oxygen demand; BOD, biochemical oxygen demand

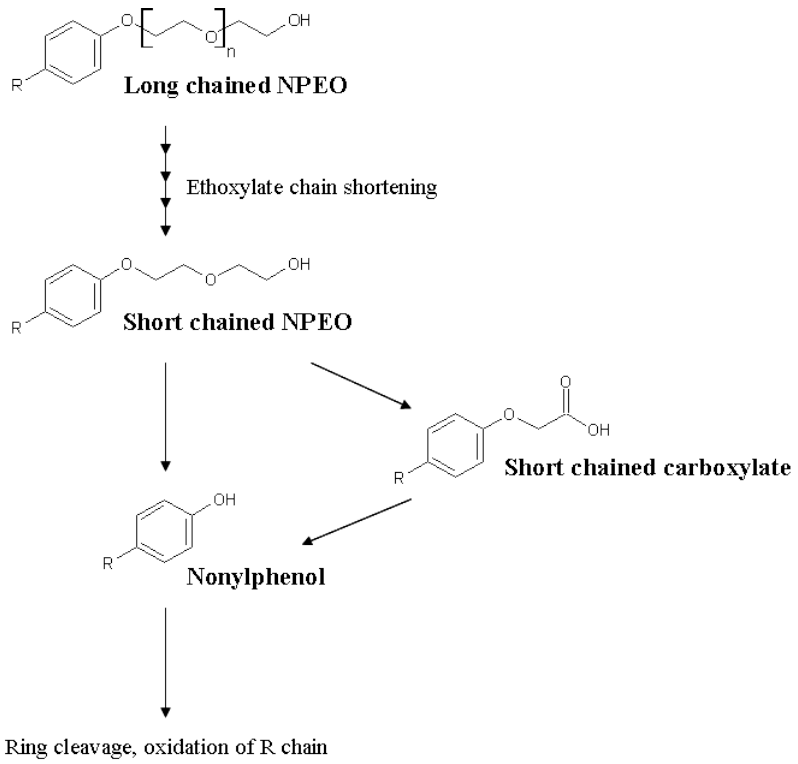


Figure S1. Proposed degradation pathway of long chained NPEOs during wastewater treatment, adapted from (Warhurst, 1994; Soares *et al.*, 2008)

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