The final publication is available at

http://doi.org/10.1039/c7ew00382j

Additional Information

Fate of Endocrine Disruptor Compounds in an Anaerobic Membrane BioReactor (AnMBR) coupled to an activated sludge reactor

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Abstract:

The occurrence and fate of three groups of micropollutants, Alkylphenols, pentachlorophenol and hormones, were studied in a pilot plant consisting of an Anaerobic Membrane BioReactor (AnMBR) coupled to an activated sludge reactor (University of Cape Town configuration - UCT). Under anaerobic conditions, the octylphenol and technical-nonylphenol soluble concentrations increased producing negative degradation ratios (i.e., -175 and -118%, respectively). However, high 4-n-nonylphenol and bisphenol-A degradation ratios (92 and 59% for 4-n-nonylphenol and bisphenol-A, respectively) as well as complete pentachlorophenol, estrone, 17β-estradiol and 17α-ethinylestradiol removal were observed. Under aerobic conditions (UCT), octylphenol, technical-nonylphenol, 4-n-nonylphenol and bisphenol-A degradation ratios were higher than 84%. The AnMBR thus removes a high proportion of 4-n-nonylphenol, pentachlorophenol, estrone, 17β-estradiol and 17α-ethinylestradiol, but requires a later post-treatment process (such as UCT) to improve bisphenol-A, octylphenol and technical-nonylphenol degradation ratios. The overall AnMBR-UCT degradation ratios were 48% and 70% for octylphenol and technical-nonylphenol, respectively, and higher than 97% for 4-n-nonylphenol and bisphenol-A. AnMBR produced a higher micropollutant accumulation in the sludge than UCT: removal by adsorption in the AnMBR process was between 0.5 and 10%, and less than 0.5% in the UCT process. The combination of AnMBR and UCT technologies produces an effluent stream with low concentrations of micropollutants.

Keywords:

Activated sludge; anaerobic membrane bioreactor; alkylphenols; endocrine disruptor; hormones; urban wastewater.

1. Introduction

Anaerobic Membrane Bioreactors (AnMBR) are considered as a sustainable approach for low-strength wastewater treatment since they involve a lower environmental impact than aerobic processes in many aspects, such as net balance of greenhouse gas emissions as well as the possibility of total nutrient recovery from urban wastewaters.[1]

However, besides the aforementioned classical pollutants, other substances now found at trace levels in wastewaters must be taken into account when assessing effluent water quality.

Some of the above-mentioned trace-level chemicals, known as endocrine disruptor compounds (EDCs), are able to disrupt the endocrine system. EDCs are of global concern due to their widespread occurrence, persistence, bioaccumulation and potential adverse effects on the ecosystem and human health.

Among the great variety of non-natural substances that can now be found in water, the Alkylphenol Polyethoxylates (APEOs), its metabolites, Alkylphenols (APs), some phenolic derivatives and hormones point out in Directive 2013/39/EU[2] are being widely studied, due to their potential to act as EDCs and affect the normal functioning of endocrine systems of some organisms. These micropollutants and other EDCs have been studied in surface waters[3,4] and WWTPs.[5,6,7,8]

APEOs are a group of compounds widely used as non ionic surfactants in industrial, agricultural and domestic applications. During the wastewater treatment process APEOs can be degraded to APs: octylphenol (OP) and technical nonylphenol (t-NP), which are more active and lipophilic than the APEOs themselves. Although 4-n-nonylphenol (4-NP) is an AP, it is not a metabolite of APEOs, its occurrence therefore being infrequent in the environment.[9,10]

APEO removal has been studied by several authors in Conventional Treatment Plant (CTP) or Membrane Bioreactor (MBR) operating configurations. González et al. (2007) studied

the removal of APEOs using an aerobic MBR configuration working in parallel with an anaerobic CTP. The MBR obtained better removal results than the CTP. Similarly, several authors have concluded that aerobic MBR systems are better at removing APEOs but need an anaerobic step for their complete biodegradation.[12] Other authors studied the fate of APEOs in the anaerobic digestion process and concluded that alkylphenol mono- and di- ethoxylates are degraded to APs and accumulated in the sludge.[13,14] This was confirmed by the increased concentration of OP and t-NP in the anaerobic digested sludge, which indicates that anaerobic environments enhance the accumulation of nonylphenols.[5,6,15,16] Sato et al. (2003) and John and White (1999) have proposed possible biodegradation mechanisms of Octylphenol Polyethoxylates (OPEOs) and Nonylphenol Polyethoxylates (NPEO) under aerobic conditions, whose main feature is the removal of a glyoxylic acid group for OPEO and an acetaldehyde group for NPEO in every de-ethoxylated step and the hydroxyl group is transformed into carboxyl end-groups. On the other hand, under anaerobic conditions only, there is a gradual shortening of the ethoxylates chain. The biodegradability of 4-NP is higher than the rest of the APs studied because of the linear alkyl chain. The linear chain shows a secondary carbon attached to the C4 position of the aromatic ring, which is less resistant than the quaternary carbon of t-NP.[19,20]

Regarding Bisphenol-A (BPA), several authors confirmed that BPA presents poor biodegradation under anaerobic conditions in both suspended and soluble fractions,[21] whereas its biodegradability increases under aerobic conditions.[22] Several studies found that Pentachlorophenol (PCP) might disrupt the thyroid endocrine system,[23,24] and it can be removed under both aerobic conditions, in CTP systems, and anaerobic conditions.[25] Among the hormones, estrone (E1) and 17β-estradiol (E2) hormones are easily biodegradable due to E1 and E2 are natural hormones, and 17α-ethinylestradiol (EE2) hormone is more resistant to biodegradation due to EE2 source is synthetic. These hormones have been biodegraded in

WWTPs with removal ratios higher than 80% for E1 and E2, however EE2 only achieved removal ratios over 60%.[26,27]

The APs, PCP and hormones described above are hydrophobic organic pollutants, and in aquatic environments tend to accumulate in the solid phases, such as sediments, underwater fauna or WWTP sludge. However, the magnitude of this accumulation depends on analytes and solid phase properties. Both the aqueous and solid phases must therefore be considered in order to study the fate of these micropollutants. Most publications describe methods of micropollutant analysis in the aqueous phase, but less information has been reported for the analysis in the solid phase.[28,29] The widespread use of non-ionic surfactants and hormones means they are very likely to be found in municipal and industrial wastewaters, whereas PCP is more frequent in wastewater with a strong industrial component. In literature, the reported influent wastewater concentrations of the target compounds range from 14 to 1000 ng/L.[22,29] All in all, the behaviour of these substances and their metabolites must therefore be studied in WWTPs in order to analyse their biological or physical removal, to ensure effluent discharge standards and to improve the quality status of the receiving waters.

As previously stated, the AnMBR-UCT coupled process might be a sustainable approach for low-strength wastewater in terms of organic matter and nutrient removal, but the behaviour of micropollutants in this new system should be also assessed. No description of micropollutants behaviour has been provided yet in this novel AnMBR-UCT process. The aim of this work was to study the removal and fate of eight micropollutants (OP, t-NP, 4-NP, BPA, PCP, E1, E2 and EE2) in an AnMBR-UCT coupled process.

2. Materials and methods.

2.1. Pilot plant description and wastewater characteristics

Figure 1 shows the flow diagram of the AnMBR-UCT pilot plant used in this study, located in the Carraixet WWTP (Valencia, Spain). The AnMBR-UCT pilot plant was designed for treating a maximum flow-rate of 1200 L/h and it consists of an anaerobic membrane bioreactor followed by a post-treatment operating in UCT configuration.

The AnMBR plant description was shown in Giménez et al. (2011). The AnMBR module was operated at an average sludge retention time (SRT) of 40 ± 5 d and a hydraulic retention time (HRT) of 19 ± 6 h. The anaerobic bioreactor worked at suspended solid concentrations around 16.1 ± 1.8 g/L. Temperature and pH were varied between on-line measurements were taken from AnMBR reactor, the average temperature was 22 ± 3 ºC and the average pH was 7.3 ± 0.3.

The UCT plant description was shown in Sánchez-Ramírez et al. (2015). The UCT post-treatment system was fed with the effluent of the AnMBR and was operated at an average SRT of 18 ± 2 d and dissolved oxygen concentration between 1.5 and 2.0 mg/L. The average temperature was 20 ± 3 ºC and the average pH was 6.8 ± 0.3.

The AnMBR-UCT system was operated in steady-state under the operating conditions described above for 250 d approximately. The average influent wastewater characteristics of the pilot plant during the studied period were: 600 ± 140 mg COD/L, 74 ± 13 soluble mg COD/L, 470 ± 50 mg BOD/L, 34 ± 8 mg NH4-N/L, 4.2 ± 1.0 mg PO4-P/L, 106 ± 13 mg SO4-S/L, 9 ± 7 mg CH3COOH/L of Volatile Fatty Acids and 350 ± 30 mg CaCO3/L of Alkalinity.

An initial screening campaign was carried out to select the organic micropollutants of interest. The most relevant/abundant organic micropollutants found in the influent

wastewater were 4-NP, t-NP, OP, BPA and PCP. Moreover, three hormones (E1, E2 and EE2) with low frequency of occurrence were selected in the study.

2.2. Reagents and solutions

4-NP (CAS Number 104-40-5) and t-NP (CAS Number 84852-15-3) were purchased from Riedel-de Haën (Seelze, Germany), OP (CAS Number 140-66-9), BPA (CAS Number 80 05 7), PCP (CAS Number 87-86-5), E1 (CAS Number 53-16-7) and E2 (CAS Number 50-28-2) were purchased from Sigma-Aldrich (Steinheim, Germany). EE2 (CAS Number 57-63-6) was purchased from Fluka Biochemika (Steinheim, Germany). All the reagents were of analytical grade. SM1 shown water solubility and log Kow of each compound.

Methanol was purchased from Merck (Darmstadt, Germany). Pure water was obtained by means of a Milli-Q water purification system (Millipore, Billerica, MA, USA). Acetonitrile was purchased from J.T. Baker (Deventer, The Netherlands). Florisil (60-100 mesh) was obtained from Aldrich (Steinheim, Germany) and octadecylsilica bonded phase (Bondesil C18 40 μm) was obtained from Varian (Harbor City, CA, USA).

The stock solutions of standards were prepared in methanol up to a maximum concentration of 1000 mg/L. The more dilute solutions were prepared from stock solutions directly in water up to a maximum concentration of 1 mg/L. All solutions were kept at 4 ºC until use.

2.3. Sampling and storage of samples

In order to study the distribution and fate of the studied micropollutants, a five-point sampling campaign was carried out. These points were located at: the influent of the AnMBR system, the membrane biological reactor, the effluent of AnMBR, which corresponds with the UCT influent, the biological reactor of the UCT system (sample was collected from the aerobic section) and the secondary settler effluent. 21 samples, in each sample point, were taken.

Influent and effluent water samples from the studied pilot plant were collected in brown glass bottles as 24 h composite samples. Samples were centrifuged at 9000 rcf for 10 min (Eppendorf Centrifuge 5804, Brinkman Instruments, Westbury, NY), in order to separate the supernatant from the suspended fraction. The soluble fractions were analysed by GC/MS on the day that the samples were taken. The suspended fraction was frozen at -80 ºC, dehydrated by freeze-drying and then stored in a dry environment.

2.4. Analytical methods

Solid Phase MicroExtraction (SPME) was used as the pre- concentration technique to determine the analytes of interest.[31,32] The pre-concentrate was analysed by Gas Chromatography coupled to a Mass Spectrometry detector (GC/MS).

The micropollutant analyses were carried out at room temperature in both the soluble fraction and the suspended fraction. The GC/MS analyses were carried out in selected ion monitoring (SIM) mode. In all assays, polyacrylate (PA) fibres were used (Sigma-Aldrich, Steinheim, Germany). The SPME device was placed at the GC interface and the target compounds were desorbed from the fibre under static.

The method for soluble fraction analysis was described in Moliner-Martínez et al. (2013), and the method for suspended fraction analysis was described in Campíns-Falcó et al. (2008). SM2 shows the complete outline of the analytical procedure for composite samples.

2.5. Chromatographic conditions

All analyses were performed on a GC/MS system, consisting of a 6890 GC and a 5973 MSD (Agilent, San José, USA). The capillary column was a fused-silica HP-5ms Ultra Inert (30.0 m, 250 µm I.D., 0.25 µm film thickness) (Agilent, San José, USA). Helium was used as carrier gas at a flow of 1.0 mL/min. The transfer line was held at 280 ºC, and the ion source at 250 ºC. The

MS worked in selected-ion-monitoring (SIM) mode and the electron impact energy was set to 69.9 eV.

The gas chromatograph was operated in splitless mode and the injection port temperature was held isothermally at 280 ºC. The oven temperature program used was as follows: initial temperature of 50 ºC, 30 ºC/min to 140 ºC, held for 1 min, 20 ºC/min to 280 ºC, held for 4 min, 30 ºC/min to 310 ºC, held for 2 min, for a total run time of 19 min.

2.6. Analytical Parameters

The micropollutant retention time was determined using up to 5 µg/L of each aqueous standard solution. The mass spectrometer was operated in full scan mode and the working range was set up from 100 to 300 m/z in order to determine the characteristic ions and the relative abundance of each compound. Characteristic ions were used for sample quantification, as the GC/MS worked in SIM mode. The mass spectra of the studied micropollutants can be observed in Supplementary Material SM3. The SIM mode analysis was used to determine the quality assurance parameters such as detection and quantification limits, precision and linearity. The analytical procedure was validated in terms of linear dynamic range and precision (Relative Standard Deviation, RSD).

The limit of detection (LOD) and the limit of quantification (LOQ) were determined experimentally as the lowest concentration giving a chromatographic peak three times the signal/noise ratio and ten times the signal/noise ratio, respectively. Supplementary Material SM4 and SM5 show LOD, LOQ, intra-day precision (RSD), calibration line parameters, correlation coefficient and lineal range for soluble and suspended fraction, respectively. The LOD values ranged from 2 to 600 ng/L for the soluble fraction and from 20 to 1000 ng/kg for the suspended fraction.

The determined regression coefficients for the soluble and suspended fraction were higher than 0.99. The precision of the methods for the soluble and suspended fractions were evaluated by the RSD statistical parameter. The RSD values of the aqueous analytical procedure were obtained by spiking aqueous samples with 1.0, 5.0, 10.0, 5.0, 5.0, 20.0, 20.5 and 20.0 µg/L for OP, 4-NP, t-NP, BPA, PCP, E1, E2 and EE2, respectively. Satisfactory RSD values equal or lower than 20% were obtained in all cases. The RSD values of the suspended fraction analytical procedure were obtained by spiking 1.00±0.01 g of free micropollutant suspended fraction with 0.4, 4.0, 2.0, 10.0, 10.0, 8.1, 8.1 and 8.1 µg/kg for OP, t-NP, 4-NP, BPA, PCP, E1, E2 and EE2, respectively. For the soluble fraction, satisfactory values equal or lower than 20% were obtained in all cases for the suspended fraction.

2.7. Mass Balance

The mass fluxes of each micropollutant in influent (F_I), effluent (F_E) and purge (F_p) were determined according to Equations (1), (2) and (3), respectively. The generation of each process (G) was determined by Equation (4). The removal ratios by adsorption (R_{Ads}) and degradation (R_{Deg}) were evaluated with Equations (5) and (6), respectively.

$$F_I = Q_I \cdot \left(S_I + (TSS_I \cdot X_I) \right) \tag{1}$$

$$F_E = (Q_I - Q_P) \cdot \left(S_E + (TSS_E \cdot X_E) \right) \tag{2}$$

$$F_P = Q_P \cdot \left(S_R + (TSS_R \cdot X_R) \right) \tag{3}$$

$$G = F_I - F_E - F_P \tag{4}$$

$$R_{Ads} = 100 \cdot \frac{\sum_{i=AnMBR,UCT}(Q_P \cdot SST_R \cdot X_R)_i + \sum_{i=AnMBR,UCT}(Q_E \cdot SST_E \cdot X_E)_i}{F_I} \tag{5}$$

$$R_{Deg} = \frac{G}{F_I} \cdot 100 \tag{6}$$

where F is the mass flux of the micropollutant (μg/d), Q is the work flow (L/d), S is the

micropollutant concentration in the soluble phase (μg/L), X is the micropollutant concentration

in the suspended phase (μg/kg) and TSS is the total suspended solids concentration (kg/L)

(Subscript *R* refers to AnMBR or UCT reactor).

3. Results and discussion

3.1. Occurrence of micropollutants in the soluble fraction

The fate of EDCs in the pilot plant was studied for a period of ten months. Figure 2 (a

and b) show the average concentrations of micropollutants in soluble fractions along the five

sampling points.

The OP and t-NP soluble concentrations increased 160±20% and 130±50%,

respectively, under anaerobic conditions. However, during aerobic conditions the OP and t-NP

soluble concentrations were reduced by 88±12% and 93±6%, respectively. As indicated by the

literature, degradation of APEOs is generally believed to start with a shortening of the

ethoxylate chain under both aerobic and anaerobic conditions.[9,11,35] Thus, the anaerobic

step caused APEO degradation, which increased the OP and t-NP concentrations (main

metabolites of APEOs). On the other hand, in the aerobic step the OP and t-NP concentrations

were seen to decrease, so that the combination of aerobic and anaerobic conditions caused

the net removal of APs (OP and t NP). In this case, the anaerobic/aerobic coupled process gave

rise to a reduction in the OP and t-NP concentrations of 70±30% and 80±20%, respectively,

showing that the AnMBR-UCT process does not completely remove OP and t-NP from the

wastewater.

The observed pattern for 4-NP showed that both anaerobic and aerobic treatments favoured the degradation process of this micropollutant. The anaerobic step showed considerable but incomplete removal (higher than 92±5%), whereas the aerobic step obtained its total removal from the wastewater. This result is attributed to the fact that 4-NP is not a metabolite of APEOs[9,10] and also because its linear chain makes 4-NP more degradable.[19,20]

Figure 2 (a) also shows that only 60±20% BPA had been removed under anaerobic conditions, whereas it was completely eliminated under aerobic conditions.

EE2 was detected at concentrations ranging from LOD to LOQ in the AnMBR influent. During the study, the EE2 was completely removed in the AnMBR process.

Finally, the observed concentrations of PCP, E1 and E2 were around LOD in the soluble fraction during the entire study period. Several authors have pointed out that PCP, E1 and E2 have high degradation ratios in both aerobic and anaerobic conditions.[25,36].

3.2. Occurrence of micropollutants in the suspended fraction

Figure 2 (c and d) shows the average concentrations of the micropollutants determined in the suspended fraction at the different sampling points. In general, OP, t-NP, 4-NP and BPA were retained in the suspended fraction and retention was higher under anaerobic than aerobic conditions.

As previously stated, anaerobic conditions enhance the APEO de-ethoxylation process, and raise OP and t-NP concentrations in the soluble fraction. The increased OP and t-NP concentrations in the soluble fraction also increase the concentration gradient between the soluble and suspended fractions, improving the adsorption onto digested anaerobic sludge. Moreover, the high potential of bioaccumulation (log Kow for OP and t-NP were 4.9 and 5.7, respectively) enhances the adsorption onto the suspended fraction. Under aerobic conditions,

APEO degradation did not occur, while OP and t-NP did undergo degradation. Hence, the difference of concentration between the soluble and suspended fractions due to partitioning is lower than under anaerobic conditions.

The concentrations of the 4-NP and BPA micropollutants in the suspended fraction were higher in anaerobic than aerobic sludge, since the concentrations of these micropollutants in the soluble fraction were higher in anaerobic conditions.

E1, E2, EE2 and PCP were not detected in the suspended fraction, due to the low concentration of these compounds in soluble fraction, which did not allow mass transport from the soluble to the suspended fraction.

In general, the results indicate that anaerobic conditions tend to produce higher micropollutant accumulation in the sludge.

3.3. Mass Balance

Mass balances were performed in order to determine the fate and removal ratios of the micropollutants detected in the pilot plant. E1, E2, EE2 and PCP were excluded from the mass balance analysis, because E1, E2 and PCP concentrations were around LOD, and EE2 was only quantified in 20% of the processed samples, which would have made an EE2 study unrepresentative.

Two main different mechanisms must be considered when describing the removal of micropollutants in a wastewater treatment: adsorption and degradation. In order to simplify the mass balance calculations, it was considered that the total concentration of micropollutant (soluble and suspended) in the influent was available for adsorption and degradation processes.

Mass balance is based on the experimental micropollutant concentrations determined in both the soluble and the suspended fractions. The mass balance analysis was carried out under the following operating conditions: influent AnMBR flowrate (QI AnMBR) of 2800±1200 L/d, AnMBR purge flowrate (QP AnMBR) of 54±12 L/d, UCT purge flowrate (QP UCT) of 20±7 L/d, total suspended solids in AnMBR reactor (TSSR AnMBR) of 0.016±0.004 kg/L and total suspended solids in UCT reactor (TSSR UCT) of 0.00113±0.00014 kg/L.

Figure 3 shows the three systems to which the mass balance was applied, divided systems were: the AnMBR process; the UCT process and the total plant AnMBR-UCT. The inflow to the UCT was determined as the difference between the inflow and purge flow to the AnMBR (QI (UCT) = QI (AnMBR) -QP (AnMBR)).

Table 1 shows the mass fluxes, the generation parameter and the removal ratios for OP, t-NP, 4-NP and BPA.

In the AnMBR process, the generation parameter for OP and t-NP was negative, indicating that under anaerobic conditions both micropollutants increased their soluble concentration. Nevertheless, the generation parameter for 4-NP and BPA was higher than zero, indicating that an anaerobic process reduces the micropollutant concentration. Regarding degradation ratios (RDeg), it was observed that this parameter was negative for OP and t-NP, confirming that an anaerobic process increases the OP and t-NP concentration in the system. 4-NP and BPA degradation ratios were higher than zero, indicating that 4-NP and BPA are removed in an anaerobic process. The RDeg value for 4-NP was higher than 90%, highlighting the degradability of this compound. The BPA RDeg value was 60±20%, indicating a lower degradability of this compound under anaerobic conditions. The RDeg values obtained for the four micropollutants (OP, t-NP, 4-NP and BPA) in the AnMBR process indicated that post-treatment would be required to achieve a micropollutant-free effluent. In the removal by

adsorption (RAds) in the AnMBR process, the RAds values were between 0.5 and 10% for the studied micropollutants. The RAds values showed two different behaviours: moderate values ranging from 4 and 10% for OP and t-NP; and values below 1% for 4-NP and BPA. As mentioned above, the results showed that under anaerobic conditions the higher difference of concentration between the soluble and suspended fractions due to partitioning enhances adsorption onto the sludge.

The UCT mass balance results showed positive removal ratios for the micropollutants studied. The generation parameter was always higher than zero and close to the UCT influent mass flux, which shows that the UCT post-treatment achieves the removal of micropollutants. Although the degradation ratio was higher than 84% in all cases, the adsorption ratios were lower than 1%, showing that micropollutant retention in sludge during this aerobic step is not favoured. The complete removal (RDeg+RAds) of 4-NP is worth noting.

The mass balance applied to the AnMBR-UCT system shows that this combined configuration was capable of removing micropollutants. This removal process was the result of a combined mechanism of adsorption and degradation. The overall RDeg values for OP and t-NP (50±20 and 70.1±9.2%, respectively) showed that removal was not complete, due to the increased concentration of these micropollutants in the soluble fraction of the AnMBR process. 4-NP and BPA showed RDeg values higher than 91%, since these micropollutants were degraded under both aerobic and anaerobic conditions. With regard to the removal by adsorption ratio, the RAds values for OP and t-NP were between 5 and 11%, and for 4-NP and BPA were lower than 1%. These two different behaviours are attributed to the higher retention of OP and t-NP in the anaerobic sludge, due to the higher soluble concentrations in the AnMBR reactor.

4. Conclusions

The anaerobic conditions maintained in the AnMBR increased the OP and t-NP concentrations by 160±20% and 130±50%, respectively, giving rise to negative RDeg values. On the contrary, the AnMBR produced high and moderate 4-NP and BPA RDeg values (i.e., 92±5% and 60±20%, respectively).

The aerobic conditions maintained in the UCT activated sludge reactor enhanced the OP, t-NP, 4-NP and BPA removal ratios, reaching RDeg values ranging from 84±14% to 99.5±0.3%.

All in all, the AnMBR-UCT process produced partial OP and t-NP removal (RDeg values around 50±20 and 70.1±9.2%, respectively) and almost total 4-NP and BPA degradation (RDeg values higher than 91%).

The adsorption process was enhanced under anaerobic conditions. This behaviour was attributed to the fact that OP, t-NP, 4-NP and BPA soluble concentrations were higher in the AnMBR than in the UCT process, and to the high micropollutant accumulation potential (log Kow). The high degradability of PCP, E1, E2 and EE2 meant that they were completely removed in the AnMBR soluble fraction, and therefore no accumulation was observed in the suspended fractions (AnMBR and UCT digested sludge).

The results indicate that an AnMBR achieves high 4-NP, PCP, E1, E2 and EE2 removal degradation ratios, but requires an aerobic post-treatment to attain high BPA and moderate OP and t-NP degradation. The AnMBR-UCT removal process was the result of a combined mechanism of adsorption and degradation.

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Tables

Set-Up		OP (µg/L)		t-NP (µg/L)		4-NP (µg/L)		BPA (µg/L)	
		Flux	s.d.	Flux	s.d.	Flux	s.d.	Flux	s.d.
AnMBR	F_I	609.4	114.6	4790.0	1382.8	648.0	172.4	6211.3	3920.9
	F_E	1616.2	276.0	9744.2	3127.3	47.3	38.4	2422.5	832.9
	F_p	60.3	2.2	688.4	266.8	4.3	3.3	120.3	38.9
	G	-1067.1	176.6	-5642.6	2059.2	596.4	133.6	3668.5	1279.4
	R_{Ads} (%)	**4.8**	3.1	**10.4**	2.4	**0.5**	0.2	**1.2**	0.9
	R_{Deg} (%)	**-175.1**	71.7	**-117.8**	20.9	**92.0**	5.1	**59.1**	21.5

		OP (µg/L)		t-NP (µg/L)		4-NP (µg/L)		BPA (µg/L)	
		Flux	s.d.	Flux	s.d.	Flux	s.d.	Flux	s.d.
UCT	F_I	1616.2	276.0	9744.2	1989.2	47.3	2.5	2422.5	45.6
	F_E	245.8	45.1	720.1	323.5	0.1	0.1	3.1	0.3
	F_p	8.8	5.3	25.2	10.6	0.1	0.1	16.9	0.3
	G	1361.6	273.6	8998.9	1207.1	47.1	2.4	2402.6	45.0
	R_{Ads} (%)	**0.1**	0.1	**0.1**	0.1	**0.5**	0.3	**0.3**	0.1
	R_{Deg} (%)	**84.3**	14.1	**92.4**	8.1	**99.5**	0.3	**99.2**	0.1

|		OP (µg/L)		t-NP (µg/L)		4-NP (µg/L)		BPA (µg/L)	
		Flux	s.d.	Flux	s.d.	Flux	s.d.	Flux	s.d.
AnMBR-UCT	F_I	609.4	48.4	4790.0	1382.8	648.0	172.4	6211.3	3920.9
	F_E	245.8	44.3	720.1	280.7	0.1	0.1	3.1	0.3
	F_p	69.1	4.7	713.6	267.1	4.5	1.3	137.2	56.2
	G	294.5	16.9	3356.3	1291.1	643.5	174.6	6071.1	1944.9
	R_{Ads} (%)	**4.8**	2.4	**10.5**	2.5	**0.6**	0.4	**1.2**	0.2
	R_{Deg} (%)	**48.3**	19.5	**70.1**	9.2	**99.3**	1.4	**97.7**	6.2

Table 1. Mass fluxes of the sample points throughout the AnMBR-UCT pilot plant. (F_I: influent mass flux; F_E: effluent mass flux; F_P: purge mass flux; G: generation; R_{Ads}: removal ratio by adsorption and R_{Deg}: removal ratio by degradation). (s.d. stands for standard deviation).
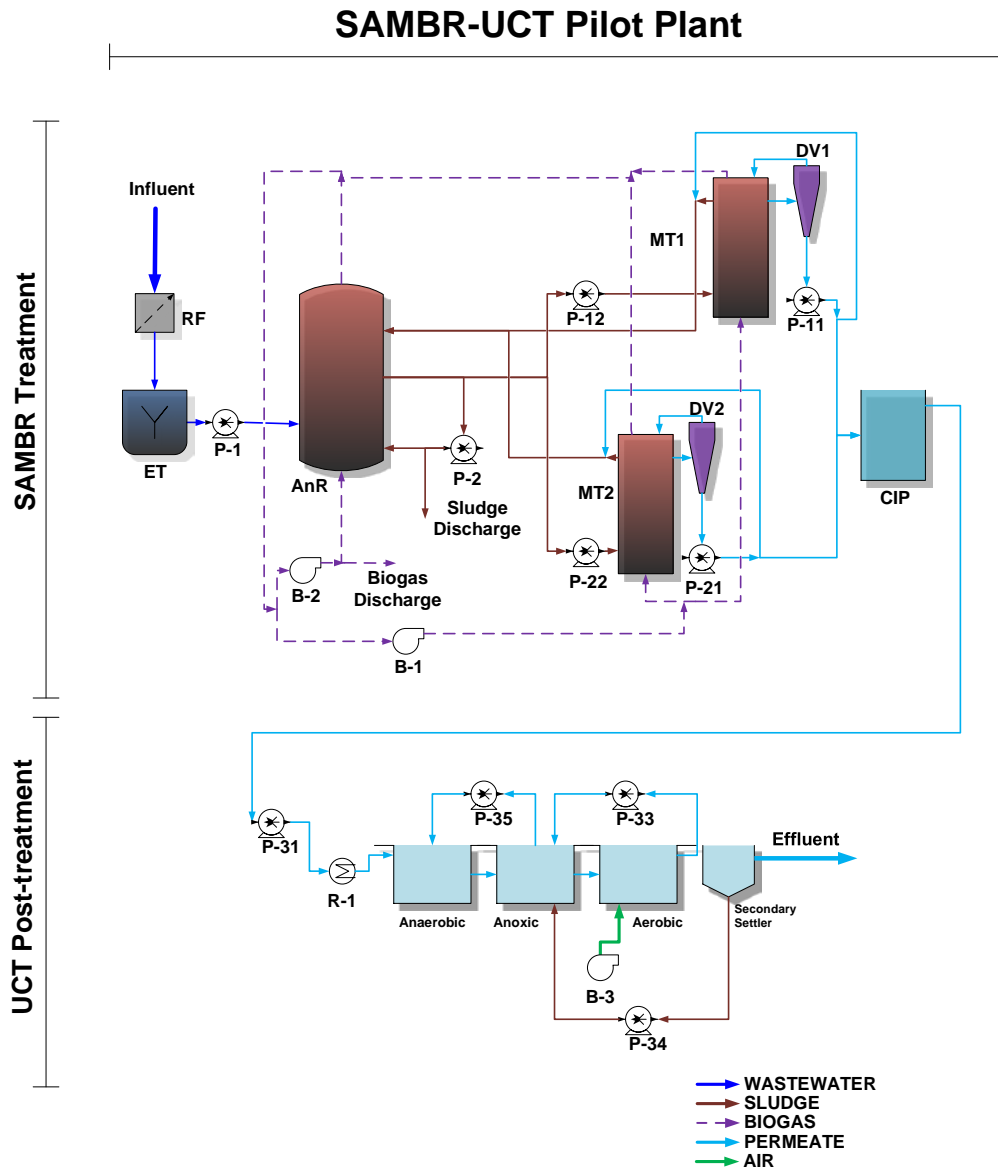
Figures

Figure 1. AnMBR-UCT pilot plant flow diagram. (Nomenclature: RF: rotofilter; R: resistance; ET: equalization tank; AnR: anaerobic reactor; MT: membrane tank; DV: degasification vessel; CIP: clean-in-place; P: pump; and B: blower).
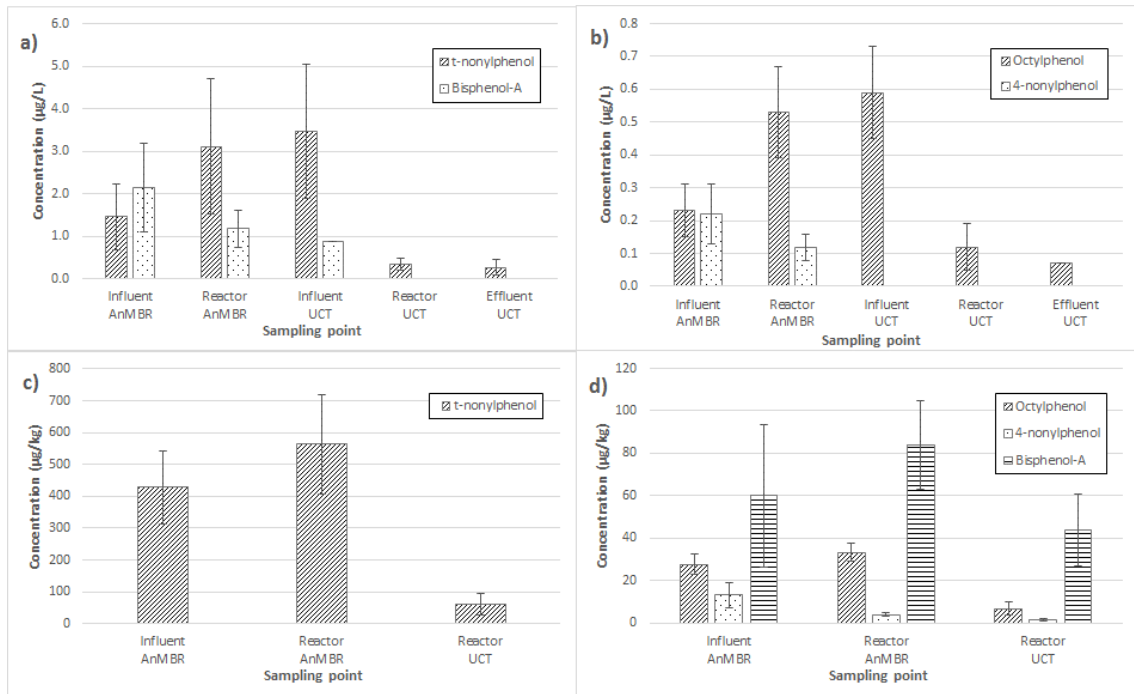
Figure 2. Average concentrations in AnMBR-UCT pilot plant sampling points; a) t-NP and BPA soluble fraction, b) OP and 4-NP soluble fraction, c) t-NP suspended fraction and d) OP, 4-NP and BPA suspended fraction. The number of samples taken in each sample point was 21.
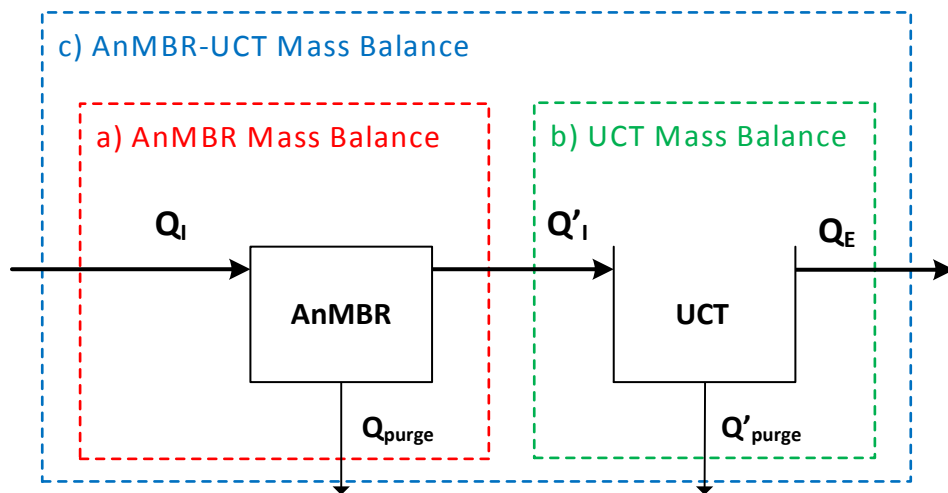
Figure 3. Systems to which mass balances were applied: a) AnMBR, b) UCT and c) AnMBR-UCT.

Supplementary material:

## Fate of Endocrine Disruptor Compounds in an Anaerobic Membrane BioReactor (AnMBR) coupled to an activated sludge reactor.

SM1. Water solubility and log Kow of studied compounds.

Compound	log K_{ow}	Water Solubility (mg/L; 20 ºC)
Octylphenol	4.9	7.0
Technical nonylphenol	5.7	4.9
4-n-nonylphenol	5.8	6.0
Bisphenol-A	3.6	120.0
Pentachlorophenol	4.7	15.0
Estrone	3.6	1.3
17β-estradiol	3.8	1.5
17α-ethinylestradiol	4.0	9.2

```
                    ┌─────────────┐
                    │  Composite  │
                    │   Sample    │
                    └─────────────┘
                           │
                           ▼
                    ┌─────────────┐
                    │  Centrifuge │
                    │  9000 rcf   │
                    │   10 min    │
                    └─────────────┘
                      ╱         ╲
                     ╱           ╲
        ┌─────────────┐      ┌─────────────┐
        │ Supernatant │      │  Suspended  │
        └─────────────┘      │  Fraction   │
                             └─────────────┘
        ┌─────────────┐      ┌─────────────┐
        │ SPME 60 min │      │ Freeze-dry  │
        │  Fibre PA   │      └─────────────┘
        └─────────────┘             │
               │                    ▼
               ▼           ┌──────────────────┐
        ┌─────────────┐    │ - 0.1 g Sample   │
        │   GC/MS     │    │ - 0.4 g C18      │
        │  SIM Mode   │    └──────────────────┘
        └─────────────┘
```

- 0.1 g Sample
- 0.4 g C_{18}

Mix during 5 min

Solid Phase Extraction:
- 0.1 g Florisil
- Mixture
- 1.2 mL ACN

Collected in a 4 mL vial

Add 2.8 mL H_2O

GC/MS SIM Mode ◄ SPME 60 min Fibre PA
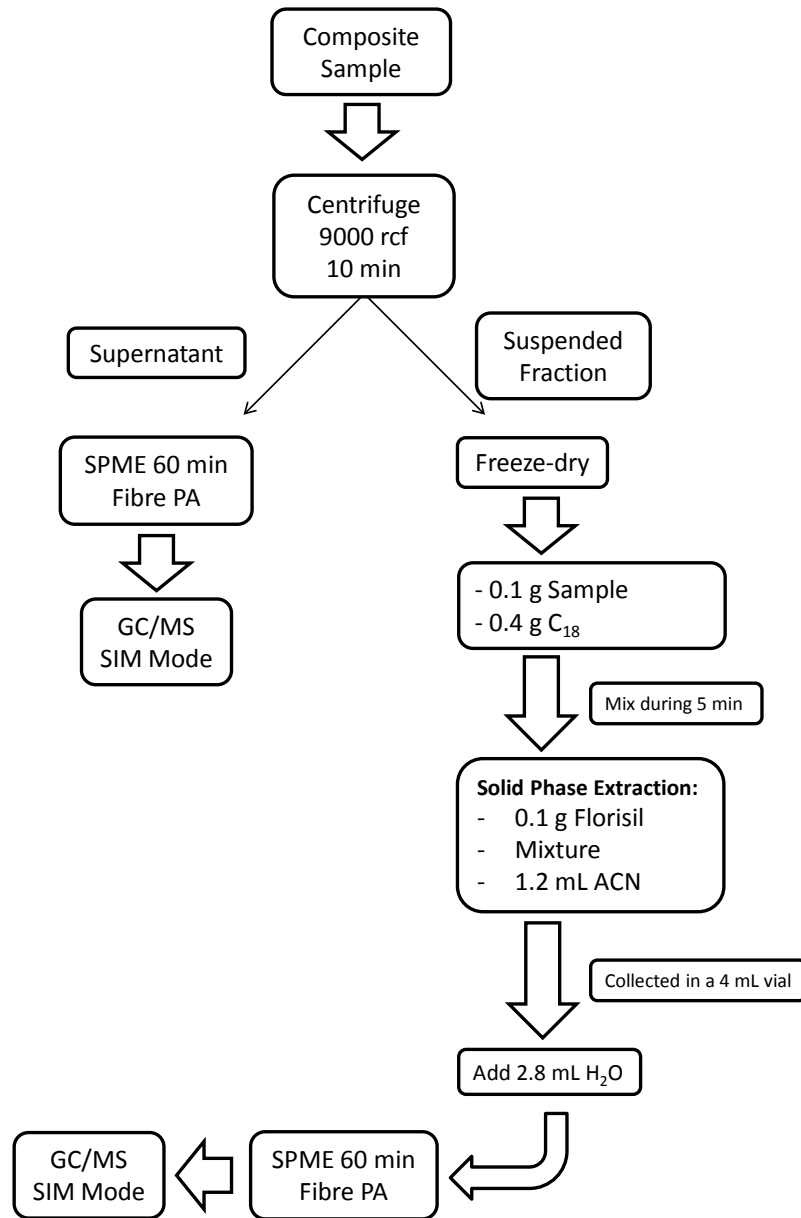
SM2. Scheme of the method used for soluble and suspended fractions analysis.

Octylphenol

4-Nonylphenol

t-Nonylphenol (R = Branched Nonyl chain)

Bisphenol-A

Pentachlorophenol

Estrone
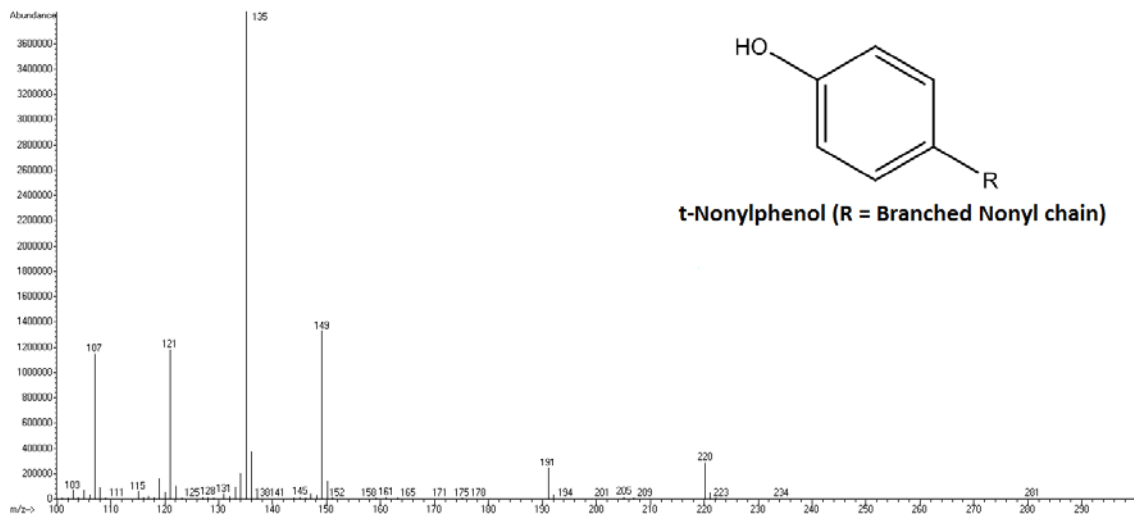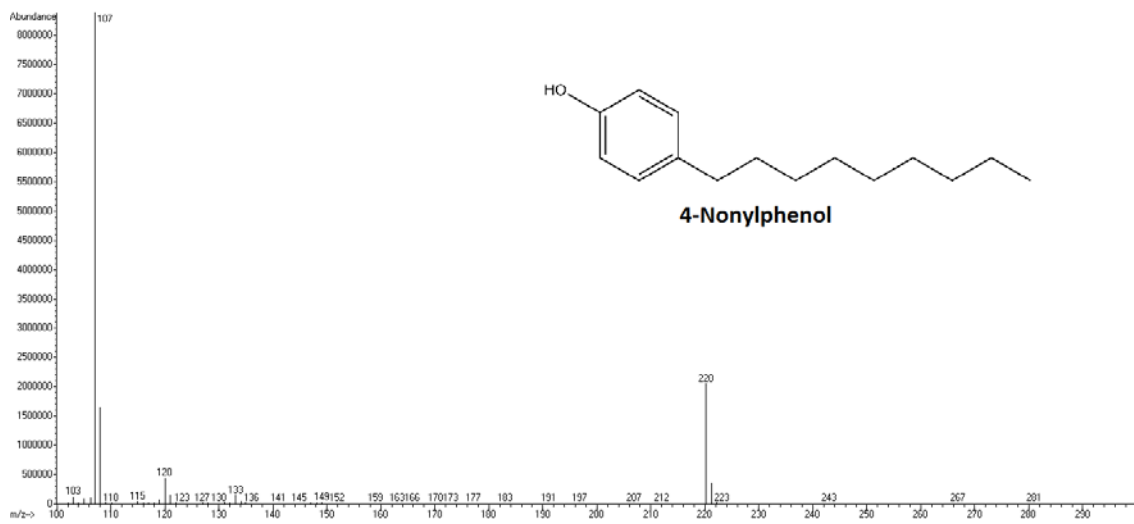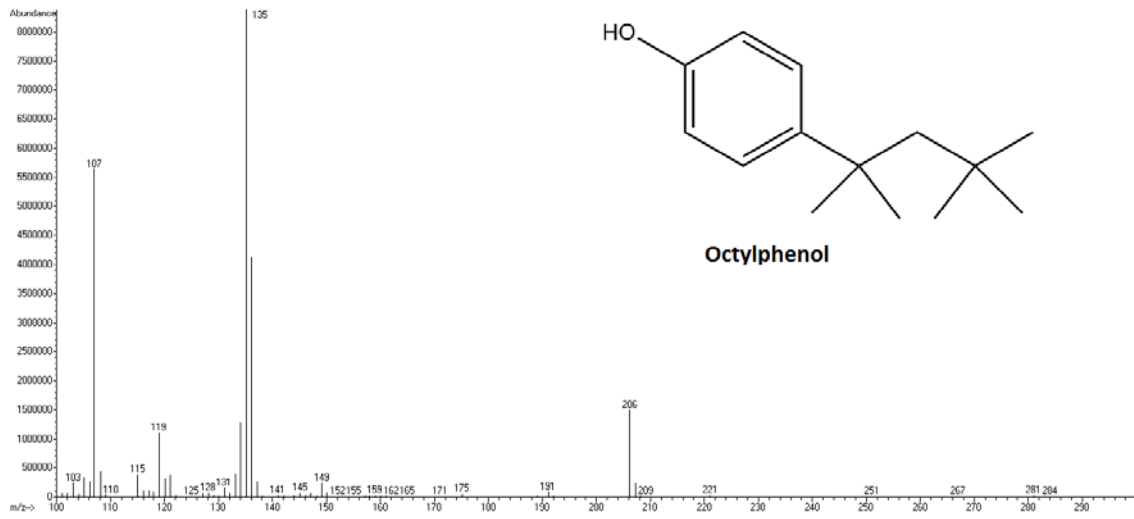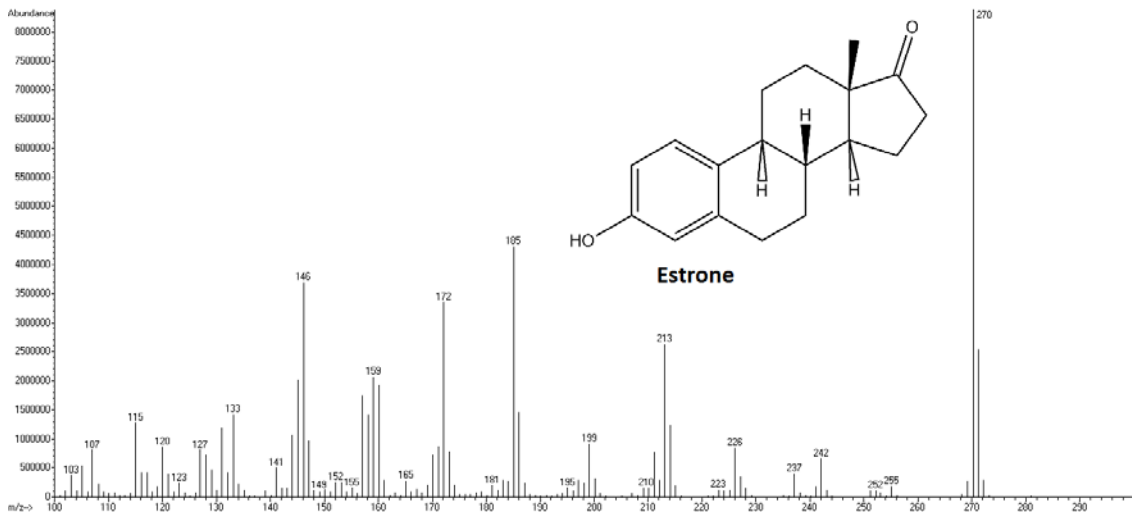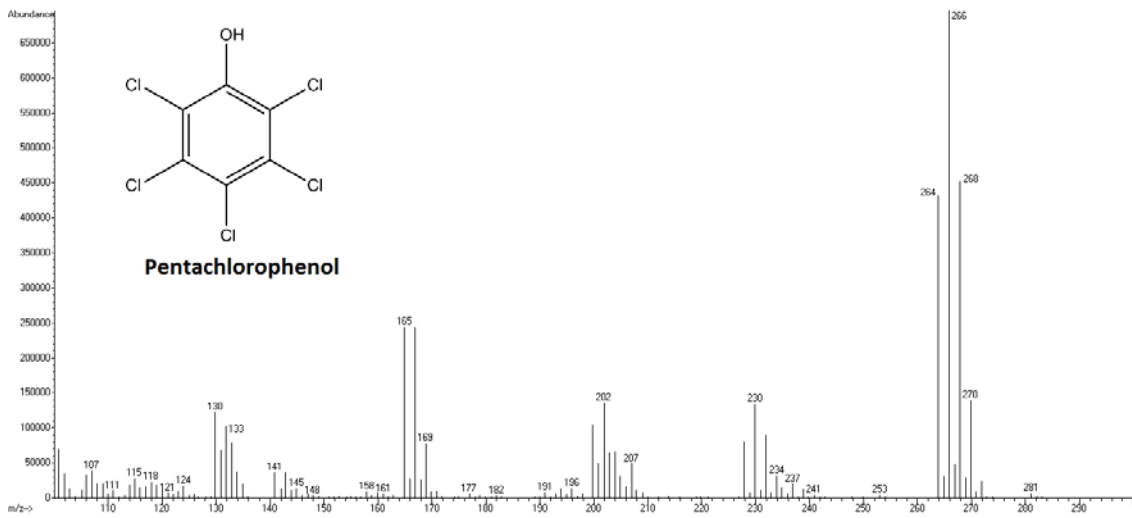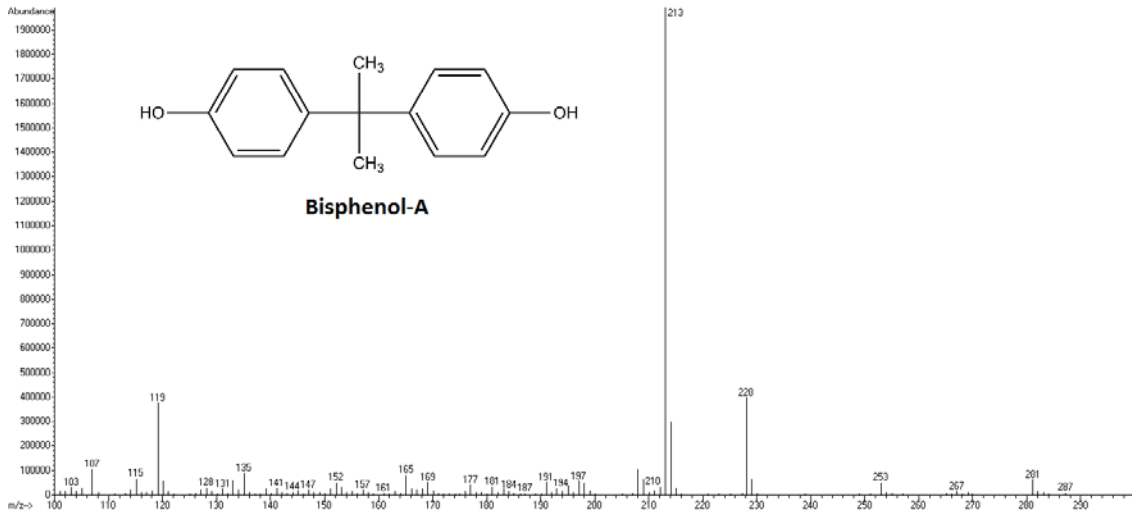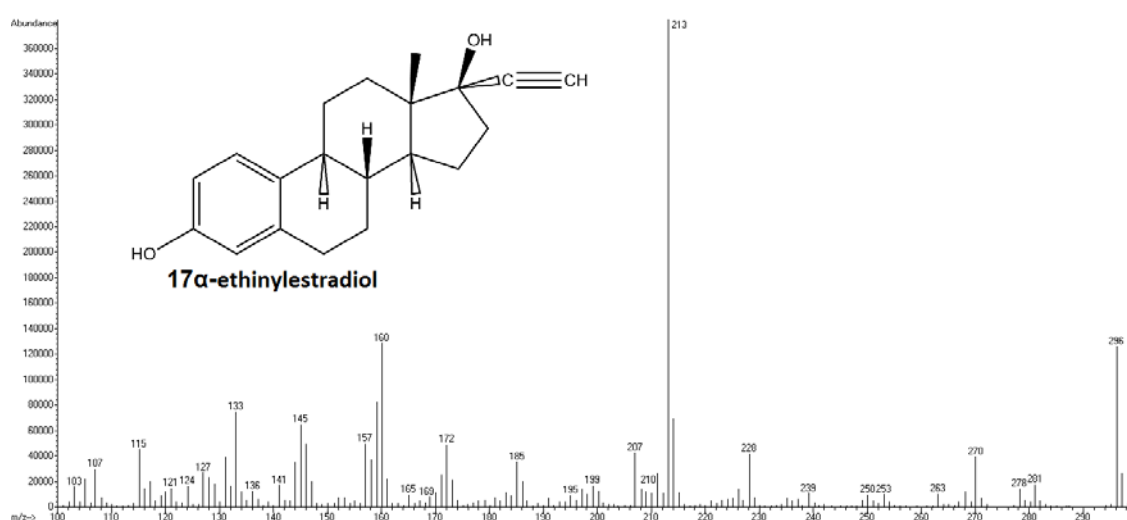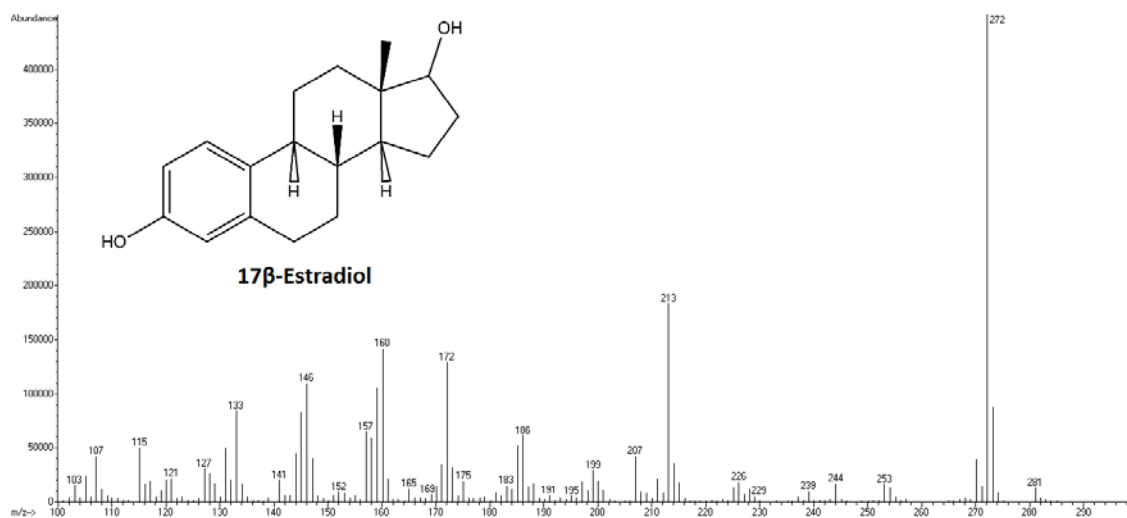
SM3. Mass spectra in scan mode (work range from 100 to 300 m/z) for OP, 4-NP, t-NP, BPA, PCP, E1, E2 and EE2 at 5 µg/L each one.

EDC	LOD (ng/L)	LOQ (ng/kg)	$a \pm s_a$	$b \pm s_b$	r^2	Range (ng/L)	RSD(%)
OP	2	7	$(-18\pm4) \times 10^5$	$(11\pm5) \times 10^3$	0.990	2 - 6000	18
t-NP	25	83	$(-6\pm2) \times 10^6$	$(5.0\pm0.2) \times 10^3$	0.990	25 - 50000	18
4-NP	8	27	$(-8\pm3) \times 10^6$	$(12.1\pm0.5) \times 10^3$	0.995	5 - 10000	20
BPA	500	1667	$(-4\pm2) \times 10^5$	$(4.4\pm0.2) \times 10^2$	0.990	500 - 50000	16
PCP	600	2000	$(-1\pm3) \times 10^6$	$(1.1\pm0.1) \times 10^3$	0.991	500 - 50000	17
E1	200	667	$(-20\pm16) \times 10^4$	74 ± 9	0.990	200 - 100000	18
E2	300	1000	$(-14\pm8) \times 10^4$	44 ± 5	0.991	200 - 100000	13
EE2	300	1000	$(-15\pm4) \times 10^4$	68 ± 2	0.993	200 - 100000	14

SM4. Analytical parameters obtained for the target analytes with SPME/GC/MS for the soluble fraction. LOD, calibration line parameters (where "a" is y-intercept and "b" is slope), correlation coefficient (r^2), linear dynamic range and intra-day precision (RSD) are shown.

EDC	LOD (ng/kg)	LOQ (ng/kg)	$a \pm s_a$	$b \pm s_b$	r^2	Range (ng/kg)	RSD(%)
OP	20	67	$(-20\pm8) \times 10^5$	$(14\pm1) \times 10^3$	0.997	20 - 6000	17
t-NP	110	367	$(-6\pm5) \times 10^6$	$(6.4\pm0.5) \times 10^3$	0.996	100 - 50000	15
4-NP	30	100	$(-11\pm4) \times 10^6$	$(16.0\pm0.8) \times 10^3$	0.990	30 - 10000	18
BPA	1000	3333	$(2.6\pm0.9) \times 10^6$	$(7\pm1) \times 10^3$	0.990	1000 - 50000	12
PCP	1000	3333	$(-5\pm4) \times 10^6$	$(1.4\pm0.1) \times 10^3$	0.994	1000 - 50000	16
E1	400	1333	$(-5\pm3) \times 10^5$	$(2.0\pm0.2) \times 10^2$	0.995	400 - 100000	15
E2	600	2000	$(-2\pm1) \times 10^5$	96 ± 8	0.996	600 - 100000	18
EE2	600	2000	$(-4\pm4) \times 10^5$	$(2.0\pm0.3) \times 10^2$	0.992	600 - 100000	19

SM5. Analytical parameters obtained for the target analytes with SPME/GC/MS for the suspended fraction. LOD, calibration line parameters (where "a" is y-intercept and "b" is slope), correlation coefficient (r^2), linear dynamic range and intra-day precision (RSD) are shown.