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Removal and fate of endocrine disruptors chemicals under lab-scale postreatment stage. Removal assessment using light, oxygen and microalgae.

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Abstract:

The aim of this study was to assess the effect of light, oxygen and microalgae on micropollutants removal. The studied micropollutants were 4-(1,1,3,3-tetramethylbutyl)phenol (OP), technical-nonylphenol (t-NP), 4-n-nonylphenol (4-NP), Bisphenol-A (BPA). In order to study the effect of the three variables on the micropollutants removal, a factorial design was developed. The experiments were carried out in four batch reactors which treated the effluent of an anaerobic membrane bioreactor. The gas chromatography mass spectrometry was used for the measurement of the micropollutants. The results showed that light, oxygen and microalgae affected differently to the degradation ratios of each micropollutant. The results showed that under aerated conditions removal ratios higher than 91 % were achieved, whereas for non-aerated conditions the removal ratios were between 50 and 80 %, except for 4-NP which achieved removal ratios close to 100 %. Besides, mass balance showed that the degradation processes were more important than the sorption processes.

Keywords:

Alkylphenol, endocrine disruptor, microalgae, wastewater treatment plant.

1. Introduction.

In recent years, several micropollutants have been detected in natural environments, mainly due to the use of manufactured products, such as surfactants, pesticides or plastic reinforcements. Some of these chemicals are able to disrupt the endocrine system. Therefore, those substances are called endocrine disruptor chemicals (EDCs). EDCs are of global concern due to their widespread occurrence,

persistence, bioaccumulation and potential adverse effects on ecosystem functioning and human health. Nevertheless, EDCs can be removed by the action of wastewater treatment plant (WWTPs) (Clara et al., 2005; González et al., 2007).

4-(1,1,3,3-tetramethylbutyl)phenol (OP) and technical-nonylphenol (t-NP) are known as alkylphenol (APs) and are degradation metabolites of alkylphenol polyethoxylates (APEOs). The annual global production of APEOs is approximately 500,000 tons, which approximately an 80% correspond to nonylphenol polyethoxylates (NPEOs), whereas the remaining 20% correspond to octylphenol polyethoxylates (OPEOs) (Lin et al., 2010). APEOs are commonly used in the formulation of a large variety of detergents, paints, lubricants, resins, and pesticides (Sharma et al., 2009). The toxicity of APEOs increases with decreasing ethoxylates chain length (Careri et al., 2003). Due to APs are more lipophilic than APEOs, these biotransformation metabolites are more toxic than APEOs. The estrogenic activity observed appeared to be confined to para- or 4 substituted compounds (Jobling and Sumpter, 1993). Mentioned estrogenic activity became stronger with the increase in the number of the alkyl carbons, being this activity maximized with a nonyl- chain. Phenol shows weak estrogenic activity, whereas anisole was completely inactive (Tabira et al., 1999). Several reports have demonstrated that these EDCs can accumulate due to high stability and lipophilicity, and thus, EDCs can affect endocrine systems in fishes and birds. Therefore, these compounds can spread to humans via the food chain (Jobling and Sumpter, 1993; Manente et al., 2011).

4-n-nonylphenol (4-NP) is an alkylphenol, but it is not a metabolite of APEOs, and thus, its occurrence in environment is infrequent (de Weert et al., 2010). Moreover, its biodegradability is higher than OP and t-NP biodegradability due to the linear chain of 4-NP presents a secondary carbon attached to C4 position of aromatic ring which is less resistant than the quaternary carbon of t-nonylphenol under both aerobic and anaerobic conditions (Corvini et al., 2006; Abargues et al., 2012; Porter et al., 2012).

BPA is principally used in the production of epoxy resins and polycarbonate plastics as a monomer. BPA can interfere in cell division mechanism, as well as mimic the female estrogen E2 (Sharma et al., 2009; Gattullo et al., 2012). Several authors confirmed that BPA presents poor biodegradation under anaerobic conditions in both suspended and soluble fractions (Ike et al., 2006), whereas its biodegradability increases under aerobic conditions (Kang and Kondo, 2002).

The Directive 2000/60/EC, also known as Water Framework Directive (WFD), is probably the most significant international legislation in the field of water from many years and its aim is to improve, protect and prevent further deterioration of water

quality across Europe (Allan et al., 2006). WFD includes and protects different kinds of water in Europe (surface water, groundwater, transitional and coastal) with the aim to achieve and ensure a good quality for all of them. WFD includes as priority substances the OP and 4-NP studied. Furthermore, Directive 2008/105/EC lays down environmental quality standards (EQS) for priority substances and certain other pollutants as provided for in WFD. The Directive 2008/105/EC establishes the extent permitted of OP and 4-NP in inland and other surface waters. So, attention must be paid on the fate of these substances in order to fulfill the WFD requirements. Furthermore, the proposal for a Directive 2011/0429 (COD) added t-NP to the list of priority substances, laying down EQS for these substances.

The removal of EDCs in WWTPs can be carried out by aerobic and anaerobic process. Several works showed that the concentrations of OP and t-NP were increased during anaerobic treatment, whereas it was observed an effective removal after aerobic activated sludge treatment (Janex-Habibi et al., 2009). Elimination of OP, t-NP, 4-NP and BPA have been studied by several authors in conventional treatment plant (CTP) or membrane bioreactor (MBR) operational configurations (Clara et al., 2005; González et al., 2007). The removal ratios obtained in the MBR showed better results than the ones reported for the CTP.

Besides, the microalgae culture can be used as removal system of EDCs. The work of Nakajima et al., (2007) showed the removal of BPA by accumulation in microalgae cells after an incubation period with microalgae culture. Other authors observed the photodegradation of BPA is enhanced by algae. The algae may produce some secretions which after irradiation can produce hydroxyl radicals that can enhance the BPA degradation (Peng et al., 2006). The OP and t-NP removal can be improved by the cyanobacteria cells, this effect was enhanced at low concentrations of OP and t-NP (Stoichev et al., 2010). Whereas, the works developed by Corvini et al., (2006) proposed a mechanism for degradation of nonylphenols with bacterial strain *Sphingomonas* sp. which initial step of degradation is an ipso-substitution.

The aim of this research was to study the removal and fate that OP, t-NP, 4-NP and BPA experience in the presence of light, oxygen and microalgae. The experimental study was designed as a postreatment stage of a Submerged Anaerobic Membrane BioReactor (SAnMBR) located in the Cuenca del Carraixet WWTP in Alboraya (Valencia, Spain). Degradation experiments combining the studied variables were carried out in batch reactors. In order to observe the fate of EDCs in the system, a mass balance was performed considering both the soluble and the suspended fraction.

2. Materials and methods

2.1. Fractional factorial design and set up reactors

In order to study the effect of light (P), oxygen (O) and microalgae (M) on the removal of micropollutants, a factorial experimental design was developed (Box and Hunter 1961). A full 2^k factorial design requires all combinations of two versions (n) of each variable (f). The total number of experiments carried out was 8 (n^f), which corresponds to three variables (light, oxygen and microalgae) and two version for each variable. The eight described experiments were reduced to five experiments (see Table 1). The removed experiments were those having the variable microalgae without variable light, because microalgae growth is inhibited under those conditions.

The experiments were carried out in batch mode, and the experimental setup was shown in supplementary Fig. S1, which consists of four Pyrex® reactors of 2.0 L of total volume, with a working volume of 1.6 L. The reactors were kept under agitated conditions in a Walk-in Ineltec climatic chamber (Tona, Barcelona, Spain), at 20 ± 1 ºC. The reactors were fed with 800 mL of a SAnMBR pilot plant effluent. The remaining 800 mL were filled with raw microalgae culture for "POM" (light, oxygen and microalgae) experiment and 800 mL of pure water for "PO" (light and oxygen), "P" (light) and "O" (oxygen) experiments.

The developed experiments under forced aeration were carried out with a compress air stream (3 bar) (Atlas Copco, ZT-37-FF, Stockholm, Sweden). The compress air was connected to a diffuser at the bottom of the reactors, so the bubbles kept the reactors agitated. The value of oxygen saturation is 8.0 mg O_2/L at 20 ± 1 ºC and 101.325 kPa (Lide, 2012). The dissolved oxygen was measured by OxiCal-SL CellOx 325 probe (WTW, Weilheim, Germany), connected to the Multi 340i meter (WTW, Weilheim, Germany). In order to maintain the pH between 7.2 and 7.5 in the microalgae culture reactor (POM), a stream of carbon dioxide was used. The pH was measured by WTW SenTix 41 probe (WTW, Weilheim, Germany), connected to Consort pH Meter model C861 (Turnhout, Belgium). Besides, the carbon dioxide stream provides a source of inorganic carbon needed for microalgae growth. Both gases, oxygen and carbon dioxide, were connected to the same diffuser.

The experiments under illuminated conditions were irradiated by four fluorescent lamps (Sylvania Grolux, United Kingdom, London) of 18 W of nominal wattage (590 mm width, 26 mm E.D.) which achieve a constant irradiation. The Grolux lamp spectrum produces high concentration of photosynthetically active radiation (PAR). PAR was measured in the illuminated surface of reactors, resulting in a value of 120 $\mu E/m^2 \cdot s$. HOBO Photosynthetic Light Smart Sensor and HOBO Micro Station Data

Logger (MicroDAQ.com Ltd, Contoocook, NH, USA) were used in order to measure the PAR.

The experiments under dark conditions were carried out with the reactors wrapped with foil and the fluorescents lamps turned off.

Every reactor was spiked with 5.6, 24.0, 16.0 and 16.0 µg of OP, t NP, 4-NP and BPA respectively. The chosen fortification corresponds to 5 times the average concentration of the SAnMBR effluent pilot plant for each micropollutant (Abargues et al., 2012).

The SAnMBR effluent used in the experiments comes from an anaerobic bioreactor pilot plant of 1.3 m^3 total volume (0.4 m^3 head-space volume) connected to two membrane tanks of 0.8 m^3 total volume each (0.2 m^3 head-space volume) which treats urban wastewater (Giménez et al., 2011). Each membrane tank includes one industrial hollow-fibre ultrafiltration membrane module (PURON® Koch Membrane Systems (PUR-PSH31), 0.05 µm pore size). The SAnMBR pilot plant was located in the Cuenca del Carraixet WWTP in Alboraya (Valencia, Spain).

The average wastewater characteristics of the SAnMBR effluent during the studied period were: 309 ± 3 mg COD/L, 54 ± 14 mg BOD/L, 54 ± 6 mg NH_4^+-N/L, 8.5 ± 1.1 mg PO_4^{3-}-P/L, 70 ± 30 mg SO_4^{2-}-S/L, 128 ± 2 mg S^{2-}/L, 12 ± 3 mg COD/L of Volatile Fatty Acids and 610 ± 110 mg $CaCO_3$/L of Alkalinity. Solids, COD, BOD, sulphate, sulphide, and nutrients were determined according to Standard Methods (APHA, 2005). The carbonate alkalinity and volatile fatty acids concentration were determined by titration according to the method proposed by WRC (1992).

2.2. Reagents, solutions and microalgae culture

All the reagents were of analytical grade. 4-n-nonylphenol (4-NP) (CAS Number 104-40-5) and technical nonylphenol (t-NP) (CAS Number 84852-15-3) were obtained from Riedel-de Haën (Seelze, Germany). 4-(1,1,3,3-tetramethylbutyl)phenol (OP) (CAS Number 140-66-9) and bisphenol-A (BPA) (CAS Number 80-05-7) were purchased from Sigma-Aldrich (Steinheim, Germany).

Ethanol was purchased from Panreac (Barcelona, Spain) and acetonitrile was purchased from Sigma-Aldrich (Steinheim, Germ any). The pure water was obtained by means of a Synergy UV purification system purchased from Milli-Q (Millipore, Billerica, MA, USA). Helium, nitrogen and carbon dioxide were purchased from Carburos Metálicos (Barcelona, Spain).

The stock solutions of standards were prepared in ethanol up to a maximum concentration of 1000 mg/L. The more dilute solutions were prepared from stock

solutions directly in pure water up to a maximum concentration of 1 mg/L. All solutions were kept at 4 ºC until use.

The microalgae used as inoculum were obtained from the walls of the secondary clarifier of the Carraixet WWTP and kept in the laboratory under semi-continuous feeding of SAnMBR effluent and with continuous illumination varying between 114 and 198 $\mu E \cdot m^{-2} \cdot s^{-1}$. Microalgae from the chlorococcales order of the Chlorophyceae class and cyanobacteria were identified as the main groups present in the inoculum (Ruiz-Martinez et al., 2012). The pH of the medium was adjusted to 7.2-7.5 by using CO_2.

2.3. Analytical Methods

Solid phase microextraction (SPME), as pre-concentration technique, was used for the determination of the interest analytes (Guillot et al., 2006; Tan et al., 2008). The pre-concentrated was analysed by gas chromatography coupled to mass spectrometry detector (GC-MS).

In order to know the fate and removal of micropollutants both soluble and suspended fractions were analysed. The final result was obtained by averaging the n (3) repetitions performed for both fractions. The analyses were performed at room temperature.

2.3.1. Soluble Fraction Analysis

The SPME procedure was carried out with 20 mL of sample, which were placed in a 20 mL clear vial screw top (Supelco, Bellefonte, PA, USA) with a magnetic stir bar (VWR International Eurolab, Barcelona, Spain). Polyacrylate fibre (Supelco, Bellefonte, PA, USA) was immersed inside the sample with constant stirring (1500 rpm) during 60 min. The fibre was immediately removed and placed in the injection port of the GC-MS for micropollutants desorption during 15 s. The described method was adapted from a previous work (Moliner-Martinez et al., 2013).

2.3.2. Suspended Fraction Analysis

The POM reactor samples were centrifuged at 5000 rcf for 5 minutes (Eppendorf centrifuge 5804 R, Brinkmann Instruments, Westbury, NY, USA). The supernatant was discarded, whereas suspended fraction was dehydrated by freeze-dried. 0.10±0.01 g of the dehydrated sample was accurately weighted and placed in a 20 mL clear vial screw top with 2.5 mL of ACN. The standards and samples were weighed with a Mettler-Toledo microbalance XP105 Delta Range (Greifensee, Switzerland) with a resolution of 0.01 mg. The samples were sonicated in an ultrasonic bath for 60 min at 65 ºC (150 W, 40kHz, JP Selecta, Barcelona, Spain), and then were centrifuged at 5000 rcf for 5 minutes. 2.0 mL of supernatant were placed in a 20 mL

clear vial screw top with a magnetic stir bar (7 mm E.D., 20 mm width) and 18.0 mL of water were added. After that, the soluble fraction was pre-concentrated with the SPME-GC-MS technique describe above.

2.4. Chromatographic conditions

The whole analyses were performed on a gas chromatography-mass spectrometry system (GC-MS) using 6890N GC with 5973 inert MS Detector (Agilent, Palo Alto, CA, USA) equipped with a split/splitless injection port and operated by MSD Chemstation Software (Agilent, Palo Alto, CA, USA). The capillary column is a fused-silica HP-5ms Ultra Inert (30.0 m, 250 µm I.D., 0.25 µm film thickness) (Agilent, Palo Alto, CA, USA).

The transfer line is 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 is set to 69.9 eV. The GC-MS was operated in splitless mode and the injection port temperature was held isothermally at 280 ºC. The 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. Helium was used as carrier gas with a flow of 1.0 mL/min.

2.5. Analytical parameters

In order to find the characteristic ions and the relative abundance of each compound the GC-MS system was carried out in full scan mode (scan range from 100 to 300 m/z).

The selected ion monitoring (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 intra-day precision (Relative Standard Deviation, RSD).

The limit of detection (LOD) and 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.

2.5.1. Soluble fraction

Table 2 shows LOD, RSD, calibration line parameters, correlation coefficient and lineal range for the analysis of the soluble fraction. The LOD values ranged from 2 to 500 ng/L.

The determined regression coefficients were always higher than 0.99, except for 4-NP whose coefficient was 0.98. The precision for the methods was evaluated by

the statistical parameter RSD. The RSD values of the soluble fraction were obtained by spiking aqueous samples with 1.0, 5.0, 10.0 and 50.0 µg/L for OP, 4-NP, t-NP and BPA respectively of nominal concentrations. Satisfactory values equal or lower than 20 % were obtained in all cases.

2.5.2. Suspended fraction

Table 3 shows LOD, RSD, calibration line parameters, correlation coefficient and lineal range for the analysis of the suspended fraction. The LOD values ranged from 10 to 1000 ng/kg. The determined regression coefficients were ranged from 0.96 to 0.98, except for BPA, whose regression coefficient was 0.90.

The RSD values of the suspended fraction were obtained by spiking the solid sample with 0.4, 2.0, 4.0 and 20.0 µg/kg for OP, 4-NP, t-NP and BPA respectively. Spiked solid samples were immediately sonicated and analysed. The obtained results were lower than 18 % in all cases.

3. Results and discussion

3.1. Degradation of micropollutants without microalgae, oxygen and light

In order to check the spontaneous degradation of micropollutants, an experiment without the variables light, oxygen and microalgae was carried out. The observed relative concentration (C/C_0) was calculated as the concentration at specific time divided by the initial concentration of each micropollutant. The C/C_0 was kept between 90 % and 102 % showing that the natural degradation of micropollutants was not significant, due to the RSD of the method was between 13 and 20 %.

3.2. Effect of light, oxygen and microalgae on micropollutants removal

The average characteristics of the microalgae culture reactor (POM) during the experiment were 500 ± 200 mg TSS/L, 24 ± 10 mg NH_4^+-N/L, 0.27 ± 0.07 mg NO_2^--N/L, 1.1 ± 0.4 mg NO_3^--N/L and 1.4 ± 1.3 mg PO_4^{3-}-P/L. The average values of dissolved oxygen were 8.2 ± 0.5, 8.1 ± 0.3 and 8.2 ± 0.4 mg O_2/L for “POM”, “PO” and “O” experiments respectively. In order to remove the dissolved oxygen (DO) concentration in the experiment “P”, the reactor was bubbled with nitrogen during 30 min, reducing the concentration value of DO to 1.8 ± 0.9 mg O_2/L. Figure 1 shows the evolution of the micropollutants soluble concentrations during the experiments.

The results obtained for OP and t-NP, in the soluble fraction, showed that in “POM”, “PO” and “O” experiments the micropollutants were removed faster than in the “P”, experiment, achieving removal ratios above 95 % after 22.5 h (the first quarter of experiment) for aerated experiments. The removal ratios in the “P” experiment were close to 50 % for both micropollutants. The removal ratios after 90 h were higher

than 96 % for “POM”, “PO” and “O” experiments while for “P” experiment the removal ratios were lower than 66 % for OP and t-NP.

For 4-NP the four experiments achieved similar removal rates that the ones achieved for OP and t-NP. The removal ratios were higher than 90 % after 22.5 h for the “POM”, “PO” and “O” experiments and close to 75 % for the “P” experiment. After 90 h, the removal ratios were 100 % in all experiments. This behaviour can be explained due to 4-NP is easier to degrade, because this micropollutant could undergo the ipso-substitution mechanism described in literature (Corvini et al., 2006; Porter et al., 2012).

The removal results obtained for BPA showed that aerated experiments produced faster removal than the non-aerated experiment. The obtained removal ratios in aerated experiments were between 91.9 and 99.8 % after the first 22.5 h. Whereas, the non-aerated experiment showed removal ratios close to 80 %. The removal ratios after 90 h were higher than 96 % for “POM”, “PO” and “O”. However the removal ratio for “P” experiment was only 79.8 %. This value is significantly lower than the values obtained in the aerated experiments.

In general the removal observed after 90 h in the “P” experiment was less effective than in the other three experiments, except for 4-NP. The main difference among the experiments was the lower DO concentration in the “P” experiment. The DO value for “P” experiment was 1.8 ± 1.0 mg O_2/L, whereas for “POM”, “PO” and “O” experiments was 8.2 ± 0.8 mg O_2/L. This observation is in accordance with literature which suggests that the oxygen is necessary for attacking the aromatic rings (Lika and Papadakis, 2009).

3.3. Mass Balance

The mass balance was calculated once the experiments were finished (90 h). The suspended fraction, in “POM” experiment, was separated from supernatant by centrifugation and analysed.

Two methods for carrying out the mass balance are described in the literature (Carballa et al., 2007; Estrada-Arriaga and Mijaylova, 2011). The first method uses measured data for soluble and suspended fractions, whereas the second method uses the solid-water distribution coefficients to calculate the concentrations in the sludge from those measured in the soluble phase. The method based on measure data was used in this work. The mass of each micropollutant in influent (M_I) and effluent (M_E) were determined according to the equations (1) and (2). The removal in the system was studied separating the two processes: sorption (M_S) and degradation (M_D), defined in Equations (3) and (4) respectively. The removal ratios by sorption processes

(R_S) and by degradation processes (R_D) were evaluated with the equations (5) and (6) respectively.

$$M_I = V \cdot S_I \quad (1)$$

$$M_E = V \cdot (S_E + TSS \cdot X) \quad (2)$$

$$M_S = V \cdot TSS \cdot X \quad (3)$$

$$M_D = M_I - M_E - M_S \quad (4)$$

$$R_S = 100 - ((M_S/M_I) \cdot 100) \quad (5)$$

$$R_D = 100 - ((M_D/M_I) \cdot 100) \quad (6)$$

where M is the mass of EDCs (μg), V is the volume treated in batch system (L), S is the micropollutant concentrations in the soluble fraction (μg/L), X is the micropollutant concentrations in the suspended fraction (μg/kg) and TSS is the total suspended solids concentration each mass flux of "POM" reactor (kg/L). The influent is the permeate of SAnMBR system, thus the concentration of TSS in influent is zero.

Table 4 shows the mass fluxes of micropollutants and the removal ratios based on sorption and degradation processes in the soluble and suspended fractions for "POM" experiment and for the soluble fraction for the other experiments. Low values of R_S were observed in "POM" experiments indicating that accumulation in the microalgae was insignificant. The R_S values ranged from 0.06 to 4.17 % respect to the initial spiked concentration. This observation was in accordance with Nakajima et al., (2007). On the other hand, the removal of the soluble fraction was higher, being the values of R_D higher than 90 % for all the micropollutants studied.

Comparing all the experimental conditions, high total removal ratios (R_S+R_D) were achieved for t-NP. Values higher than 98 % were observed for aerated experiments, whereas for "P" experiment the total removal ratio was 65 %. The R_D for OP was higher than 98 % for aerated experiments, whereas it was closer to 50 % for "P" experiment. Although the branched chemical structure of OP and t-NP make them resistant to degradation, the aerated conditions produced removal ratios higher than 98%. The total removal ratios observed for 4-NP were higher than 99.9 % in all experiments, which evidenced that this micropollutant is highly degradable. This observation was in accordance with literature (Corvini et al., 2006; Porter et al., 2012). The BPA shows the lowest R_D for aerated experiments, which was kept between 91 and 99 %. Whereas under non-aerated conditions BPA the removal ratio was close to 80 %. The OP shows the lowest removal ratio under non-aerated conditions, thus OP seems to be the micropollutant more resistant to light.

Comparing aerated experiments, the total removal ratios obtained for OP, t-NP and BPA were higher than 96 % for “PO” and “O”, whereas for “POM” the value ranged from 96.1 to 98.8 %. This could be attributed to the microalgae were able to shield the light, therefore the low removal ratio produced by photons was partly inactive. Moreover, the micropollutants degradation under aerated conditions can be fulfilled in less than 20 h.

4. Conclusions

The total removal ratios for OP, t-NP and BPA were higher under aerated conditions than under non-aerated conditions. However, it was observed a complete removal for 4-NP in all experiments. The chemical oxidation is the key variable in the micropollutants removal and photodegradation was only effective on 4-NP. The removal by sorption was much lower than by degradation. Therefore an aerobic step should be required to remove the studied compounds from the SAnMBR effluent. Further studies must be performed in order to optimise the DO concentration. Moreover, the photosynthetic activity of microalgae in terms of DO supplier should be assessed.

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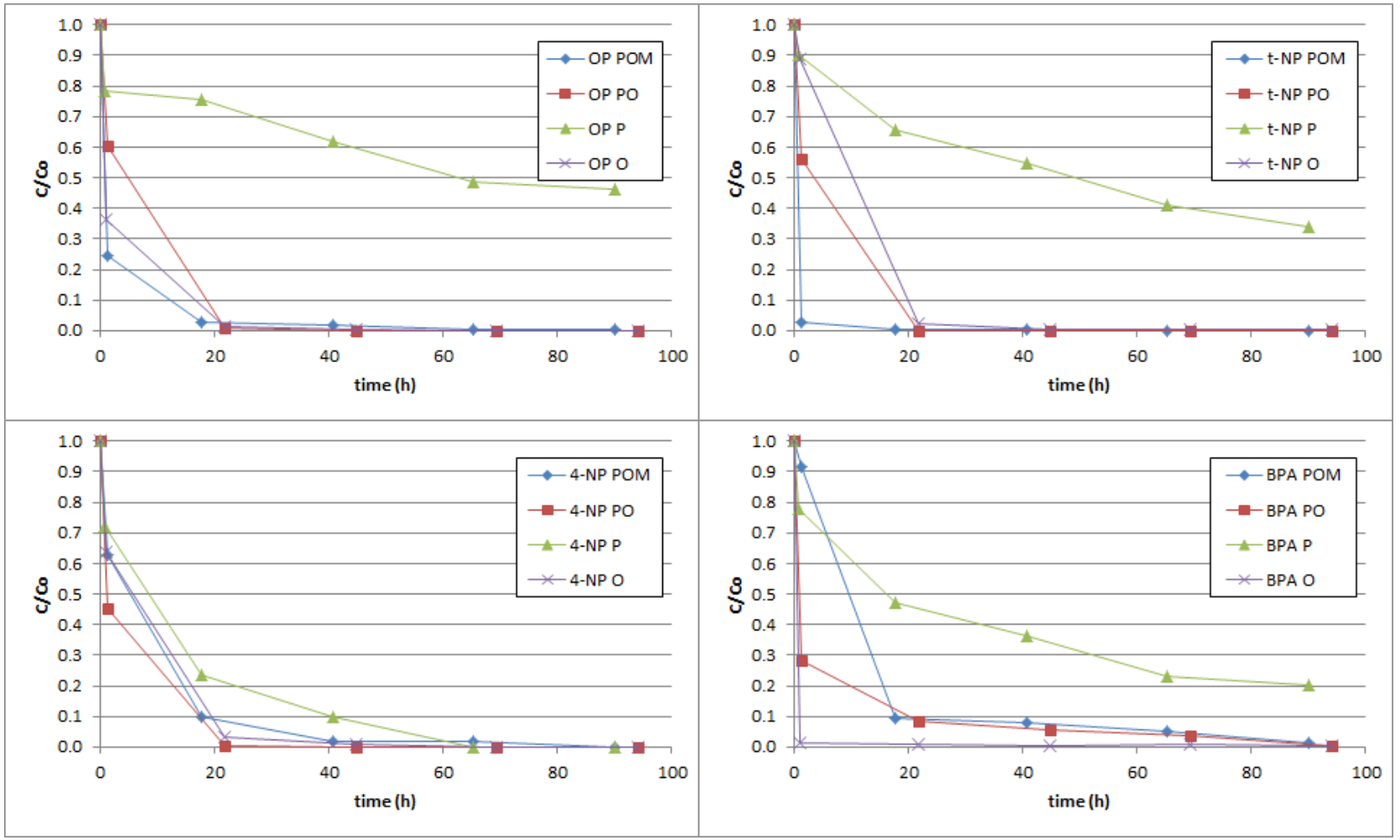
Figure captions.

Figure 1. Effect of light, oxygen and microalgae combination; ”POM” (light, oxygen and microalgae), “PO” (light and oxygen), “P” (light) and “O” (oxygen) on the micropollutants in soluble concentrations. Removal is shown through of the variation of relative micropollutants concentration with time. The initial concentrations were 3.5, 15.0, 10.0 and 10.0 µg/L for OP, t-NP, 4-NP and BPA respectively. The average exposure total time was 90 h for each experiment



518

519 Fig. 1

520 .

521 **Tables.**

522

	Value of variables			Code		
Treatment combination	**O (mg O_2/L)**	**P (µE/m^2·s)**	**M (cells/L)**	**O**	**P**	**M**
POM	8.2±0.5	120	$2{,}25 \cdot 10^{10}$	+	+	+
PO	8.1±0.3	120	0	+	+	-
P	1.8±0.9	120	0	-	+	-
O	8.2±0.4	0	0	+	-	-
1	1.8±0.9	0	0	-	-	-

523

524 **Table 1.** A typical factorial design established for three variables; oxygen (O),
525 light (P) and microalgae (M) at two levels.

526

EDC	LOD (ng/L)	LOQ (ng/L)	$a \pm s_a$	$b \pm s_b$	r^2	Range (ng/L)	RSD(%)
OP	2	6,7	$(-18\pm4) \times 10^5$	$(11\pm5) \times 10^3$	0,990	2 - 6000	18
t-NP	25	83,3	$(-6\pm2) \times 10^6$	$(5\pm0.2) \times 10^3$	0,990	25 - 50000	18
4-NP	8	26,7	$(-8\pm3) \times 10^6$	$(12\pm0.5) \times 10^3$	0,98	5 - 10000	20
BPA	500	1666,7	$(-4\pm2) \times 10^5$	$(4.4\pm0.2) \times 10^2$	0,990	500 - 50000	16

Table 2. Analytical parameters obtained for micropollutants with SPME-GC-MS for soluble fraction. LOD, LOQ, 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	10	33,3	$(-20\pm8) \times 10^5$	$(14\pm1) \times 10^3$	0,98	10 - 10000	12
t-NP	50	166,7	$(-6\pm5) \times 10^6$	$(6.4\pm0.5) \times 10^3$	0,96	100 - 100000	7
4-NP	20	66,7	$(-11\pm4) \times 10^6$	$(16.0\pm0.8) \times 10^3$	0,98	20 - 20000	17
BPA	1000	3333,3	$(2.6\pm0.9) \times 10^6$	$(7\pm1) \times 10^3$	0,90	1000 - 100000	11

Table 3. Analytical parameters obtained for micropollutants with SPME-GC-MS for suspended fraction. LOD, LOQ, 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.

540

		Mass Flux (µg/d)			
Set-up	Sample	**OP**	**t-NP**	**4-NP**	**BPA**
POM	M_I	0.73	3.13	2.08	2.08
	M_E	0.01	0.04	0.00	0.08
	M_S	0.0076	0.0696	0.0013	0.0869
	M_D	0.71	3.02	2.08	1.92
	R_S (%)	1.05	2.23	0.06	4.17
	R_D (%)	97.75	96.47	99.90	91.89
PO	M_I	0.73	3.13	2.08	2.08
	M_E	0.00	0.00	0.00	0.00
	R_D (%)	99.98	99.98	100.00	99.82
P	M_I	0.73	3.13	2.08	2.08
	M_E	0.34	1.06	0.00	0.42
	R_D (%)	53.84	65.98	100.00	79.84
O	M_I	0.73	3.13	2.08	2.08
	M_E	0.00	0.01	0.00	0.01
	R_D (%)	99.93	99.76	100.00	99.51

541 **Table 4.** Mass flux (µg/d), sorption removal ratio (R_S) and biodegradation
542 removal ratio (R_D) of the carried out operational conditions.