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Pilot-scale evaluation of ozone as a polishing step for the removal of nonylphenol from tank truck cleaning wastewater

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8

9 **Abstract**

10 The presence of nonylphenol (NP) in the wastewater of the tank truck cleaning industry is a
11 major concern because of its endocrine disruptive properties. In this paper, the use of ozone for
12 degrading NP from tank truck cleaning wastewater was investigated by operating a pilot-scale
13 biological wastewater treatment in combination with an ozonation unit. The impact of the added
14 ozonation step on the removal of NP, soluble chemical oxygen demand (sCOD) and total
15 organic carbon (TOC) was monitored over one year. sCOD and TOC removal were not
16 significantly enhanced, but the NP peak concentrations in the effluent were significantly lower
17 than those obtained after biological treatment only: a relatively low NP concentration was
18 observed, even when peak loads were present in the influent of the pilot-scale biological
19 wastewater treatment plant ($\text{influent}_{\text{bio}}$). Contrariwise, the effluent of the sole biological
20 treatment follows the peak load trends of the $\text{influent}_{\text{bio}}$. During the ozonation period, the
21 average NP concentration in the combined biological-ozone unit was $0.29 \mu\text{g/L}$, compared to
22 $1.89 \mu\text{g/L}$ for the effluent obtained after a sole biological treatment, resulting in an improved
23 average removal efficiency of 32%.

24

25 **Keywords:** ozone; nonylphenol; tank truck cleaning; nonylphenol ethoxylate, endocrine
26 disruptor

27

28

29 **1. Introduction**

30 Endocrine-disrupting compounds are increasingly recognized as a severe threat to aquatic
31 (micro-)organisms and public health (ECB, 2002). A considerable number of papers have been
32 dedicated to the endocrine disruptive effects of alkylphenol ethoxylates (APEOs) and their
33 degradation products, such as alkylphenols (AP) (Gabriel et al., 2008; Soares et al., 2008;
34 Sharma et al., 2009; Mao et al., 2012; Acir et al., 2018). Nonyl- and octylphenol compete with
35 17β -estradiol for the binding site of the oestrogen receptor due to structural similarities, hereby
36 disrupting the natural endocrine system, which could even, for example, increase the incidence
37 of breast cancer after chronic exposure (Amaro et al., 2014; In et al., 2015; Yang et al., 2015;
38 Noorimotlagh et al., 2020). Nonyl- and octylphenol ethoxylates are the most well-known
39 APEOs used as non-ionic surfactants and are applied as cleaning, washing and surface-active
40 agents in the textile industry (Olmez-Hanci et al., 2011; Araujo et al., 2018). NPEOs are
41 produced from NP by reacting phenol with mixed nonenes in a batch process. Almost 40% of
42 the produced NP is used for the production of resins and other plastics, and around 60% to
43 produce NPEOs from NP and ethylene oxide. In the Europe Union (EU) alone, it is estimated
44 that 10,000-50,000 tons of NP were produced in 2010 compared to 73,500 tons in 1997 (ECB,
45 2002; Bontje et al. 2004; AMEC, 2012). The European production of NPEOs in 2010 was
46 approximately 32,000 tons (ECHA, 2014).

47 The European Union banned this type of surfactants for domestic usage back in 2003 (Renner,
48 1997; Council Directive 2003/53/EC, 2003), but non-ionic detergents are still allowed to be
49 used for crucial industrial applications, such as manufacturing of plastics, textiles, paper and
50 metal processing (Talmage, 1994; Cornelis et al., 2005). Therefore, non-ionic surfactants are
51 still found in the produced wastewater. Nonylphenol (NP) is also present in the discharged
52 wastewater from tank truck cleaning companies. NP ethoxylates (NPEOs) are released in the
53 cleaning process of bulk tanks that contain residual NPEO loads. When this NPEO containing
54 wastewater is treated in a biological wastewater treatment plant, NP is unintentionally produced
55 (Ahel et al., 1994; Fujita et al., 2000). The presence of NP in tank truck cleaning effluent is a
56 major concern because of its endocrine disruptive properties. High concentrations of NPEOs
57 and their derivatives, such as NP, are frequently encountered in the influent of biological
58 wastewater treatment plants (influent_{bio}). As a consequence, effluents from the tank truck
59 cleaning industry contain NP. Since the biological removal efficiency for these components is
60 limited, the sole application of biological treatment is often insufficient to meet the EU limits
61 for NP (i.e., 0.3 $\mu\text{g/L}$) discharge.

62 During biological wastewater treatment, ethoxylate (EO) chain shortening of NPEOs occurs,
63 leading to the formation of short-chain NPEOs (e.g., NP1EO or NP2EO) (Ying et al., 2002).
64 The EO-chain is further oxidized into nonyl phenoxy carboxylic acids and finally NP (ECB,
65 2002; Kovarova et al., 2013). Various publications have demonstrated that NP possesses more
66 prominent endocrine disruptive characteristics than its precursor NPEO (Giger et al., 1984;
67 Servos, 1999; Ying et al., 2002; Soares et al., 2008). The most accepted reason for the increased
68 risks is the shortening of the NPEO's ethoxylate chain (Soto et al., 1991; AMEC, 2012). The
69 biodegradation of NP is low due to the branched alkyl chain and aromatic ring structure
70 (Patoczka and Pulliam, 1990). Therefore, an additional wastewater treatment technique as a
71 polishing step is required to guarantee the complete removal of NP.

72 Advanced Oxidation Processes (AOPs) are a class of wastewater treatment techniques that have
73 gradually gained recognition during the last decade. The application potential of AOPs lies
74 mainly in the treatment of wastewater containing recalcitrant organic compounds. The removal
75 of these compounds by conventional techniques, which are mainly based on aerobic or
76 anaerobic biological degradation of organic matter, is generally not achievable due to their low
77 biodegradability (Oller et al., 2011; Dewil et al., 2017; Van Aken et al., 2015, 2017). AOPs are
78 mostly used in combination with biological wastewater treatment. They are all based on the
79 oxidation of the organic compounds by OH-radicals (OH°), which are compounds containing
80 an unpaired electron. Most radicals are highly unstable and immediately react with another
81 molecule to obtain the missing electron. Various types of AOPs have been developed, including
82 ozone and hydrogen peroxide-based AOPs, which differ in the production pathway of the OH°
83 (Glaze et al., 1987; Gottschalk et al., 2009).

84 The degradation of NP and its degradation products by AOPs is treated in a few publications.
85 Ning et al. (2007a, 2007b) studied the kinetic reaction mechanism of the direct and indirect
86 oxidation by ozonation of nonyl- and octylphenol. In their work, direct and indirect oxidation
87 by ozone was investigated by adjusting the pH of the aqueous solution. The kinetic rate
88 constants of NP were reported to be $3.90 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$ and $1.1 \times 10^{10} \text{ M}^{-1}\text{s}^{-1}$ for direct and indirect
89 oxidation, respectively. Kim et al. (2005) performed electrochemical degradation experiments
90 on NP, which resulted in the formation of benzoquinone intermediates. In these publications,
91 the effect of the composition of real industrial wastewater on the removal efficiency of AOPs
92 was not investigated.

93 The focus of this paper is to investigate the removal efficiency of NP in real industrial
94 wastewater using combined biological-ozone degradation. For that purpose, a pilot-scale
95 biological wastewater treatment plant was coupled with an ozone installation and operated on-

96 site at a tank truck cleaning company to effectively optimize and analyze the decrease of the
97 NP concentration, soluble chemical oxygen demand (sCOD) and total organic carbon (TOC)
98 of the treated wastewater. In addition, the paper explores the impact of ozonation on the
99 ecotoxicity of the wastewater.

100

101 **2. Materials and methods**

102 *2.1 Pilot-scale biological wastewater treatment*

103 Two sequencing batch reactors (SBRs) with a maximum volume of 1 m³ were used in parallel
104 for the long-term pilot-scale experiments. The first SBR (SBR 1) provided the biological
105 treatment only, whereas the effluent of the second SBR (SBR 2) was additionally subjected to
106 ozonation as a polishing step. The full installation was constructed in a 20 ft shipping container
107 and was placed on-site at the premises of a tank truck cleaning company (Antwerp, Belgium).
108 This company mainly cleans tanks containing chemical loadings, including NPEO.

109 A schematic overview of the pilot-scale installation used in the tests is provided in Fig. 1. The
110 pilot-scale installation was fed with wastewater originating from the full-scale physicochemical
111 treatment unit of the company, which is the same as being fed to the full-scale biological
112 wastewater treatment. The wastewater was introduced in a buffer tank with a volume of 400 L,
113 connected to SBR 1 and SBR 2. At the start of the experiments, activated sludge (AS)
114 originating from the biological wastewater treatment of the tank truck cleaning company was
115 introduced in the SBRs. Both SBRs were operated at a mixed liquor suspended solids
116 concentration (MLSS) between 2.5 and 3 g/L to ensure good settling of the sludge. The diluted
117 sludge volume index (DSVI) was 206 mL/g, indicating a poor settleability of the sludge flocs.
118 Operational parameters of both SBR 1 and SBR 2 were chosen in such way that the same
119 substrate (S) to biomass (X) ratio (S/X) was achieved as on full-scale, i.e., 0.19.

120 An SBR cycle time of 8 h was applied, and each cycle consisted of 4 stages. During the first
121 stage (duration 300 min) aerobic treatment conditions were applied, keeping the dissolved
122 oxygen (DO) concentration of the AS between 1 and 3 mg O₂/L by intermittent aeration. 50 L
123 of fresh influent_{bio} was introduced in the bioreactor during this stage. Throughout stage 1, the
124 reaction mixture was continuously stirred using propeller mixers (ProMInent, Belgium). The
125 second stage consisted of the continuous aeration of the bioreactor. This short phase of 10
126 minutes was included to enhance the settling properties of the AS. During the settling phase
127 (stage 3, 120 min), the mixer and aeration were switched off to enable the AS to settle. The
128 cycle was completed by a discharge stage (50 min), during which 50 L of treated wastewater

129 was discharged using a floating valve. The total duration of each cycle was 8 h, resulting in 3
130 cycles performed per day.

131 The operating volume of the AS system of both SBRs was 700L (V_{AS}). Since 50 L of fresh
132 influent_{bio} was added to the AS system in each cycle, 150 L was treated daily. The hydraulic
133 retention time (HRT) of the SBR was then 4.7 days, as shown in equation (1).

$$HRT_{\text{pilot reactor}} = \frac{V_{AS}}{\frac{V_{\text{influent}_{\text{bio}}}}{d}} \approx 4.67 \text{ d} \quad (1)$$

134 The S/X ratio of the biological wastewater treatment on pilot-scale is determined by equation
135 (2):

$$\left(\frac{S}{X}\right)_{\text{pilot reactor}} = \frac{\text{kg COD}}{\text{kg VSS} \cdot \text{d}} = \frac{COD_{\text{influent}_{\text{bio}}} \times \frac{V_{\text{influent}_{\text{bio}}}}{d}}{MLSS_{\text{pilot reactor}} \times V_{AS}} = 0.21 \quad (2)$$

With:

$$COD_{\text{influent}_{\text{bio}}} = 3000 \cdot 10^{-6} \frac{\text{kg COD}}{L}$$

$$\frac{V_{\text{influent}_{\text{bio}}}}{d} = 150 \frac{L}{\text{day}}$$

$$MLSS_{\text{pilot reactor}} = 3 \cdot 10^{-3} \frac{\text{kg VSS}}{L}$$

$$V_{AS} = 700 \text{ L}$$

136

137 The calculated S/X ratio was 0.21, which approximately corresponds to the S/X ratio of the
138 company's biological wastewater treatment (0.19).

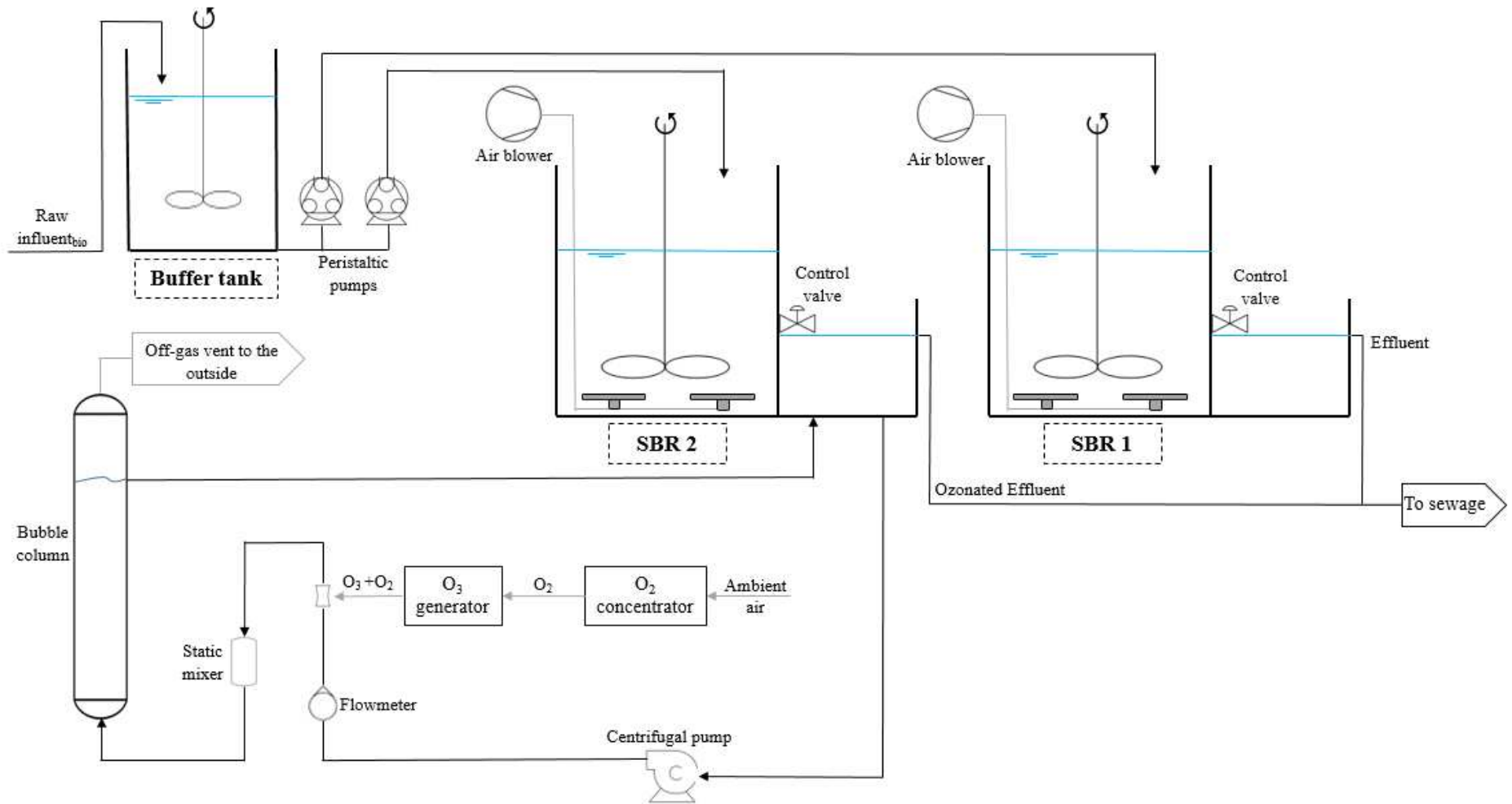


Fig. 1. Schematic representation of the pilot-scale biological wastewater treatment & ozone purification unit.

138 *2.2 Pilot-scale ozone treatment*

139 A series of lab-scale ozone experiments was performed on the effluent of the company applying
140 an ozone flow rate of 0.68 g O₃/h on 1 L samples (results not shown). The NP concentration
141 was seen to decrease from 0.56 ± 0.03 to 0.20 ± 0.02 µg NP/L after 5 min ozone treatment time,
142 resulting in an NP removal of 65%. Based on these results, an ozone treatment duration of 3 h
143 per cycle was selected for the pilot-scale ozonation unit.

144 During the pilot scale tests, the effluent of the biological treatment of SBR 2 was subjected to
145 ozonation. As shown in Figure 1, the ozonation was directly applied on the effluent via a
146 recirculation system installed over the effluent tank. The ozone system consisted of an oxygen
147 concentrator (SEP-300, Anseros) to generate a 90% oxygen containing gas stream from ambient
148 air, which was subsequently fed to an ozone generator (COM-AD-01-IP, Anseros) to produce
149 ozone via high voltage gas discharge (corona discharge). The maximum ozone production of
150 the generator was 2.75 g O₃/h at a gas flow rate of 100 L/h at 20 °C. This flow rate was kept
151 constant throughout the pilot plant operation. The ozone stream was injected in the recirculation
152 loop using a venturi-system. An in-line static mixer was installed to increase the gas-liquid
153 transfer. The ozone installation was switched on during the filling/aeration phase of the
154 biological treatment for 1 h followed by a 1 h resting period resulting in a total duration of the
155 ozonation of 3 h per cycle. After 1 h of ozonation, the wastewater was saturated with ozone.
156 The subsequent resting period allowed consuming the dissolved ozone. This way of ozone
157 injection is more energy-efficient than the continuous injection of ozone gas (Gottschalk et al.,
158 2009). The water was pumped through the ozonation unit at a fixed flow rate of 1 m³/h using a
159 centrifugal pump.

160 The injection of ozone gas increased the oxidation-reduction potential (ORP) of the treated
161 wastewater. During the settling phase of the AS, the ozone generator was switched off, resulting
162 in a decrease of the ORP to approximately 215 mV. During the filling/aeration phase, a
163 maximum ORP of 250 mV for the ozonated wastewater was recorded, which is relatively low
164 compared to the literature. If the water pH is neutral, an ORP value of 650-700 mV is expected
165 for ozone disinfection units (Suslow, 2004). A possible explanation for the low ORP values is
166 the positioning of the ORP sensor in the effluent tank instead of the ozone bubble column. The
167 effluent tank of SBR 2 contains 220 L water, whereas the bubble column has a reactor volume
168 of 10 L. Consequently, the dissolved ozone gas was diluted while entering the effluent tank.
169 Despite the sufficient water flowrate of 1 m³/h, the ozone injection time of 1 h followed by a 1
170 h resting was too short to reach the maximum ORP value in the effluent tank.

171 **2.3 Sampling**

172 During the first couple of days of pilot plant operation, a sample was taken on a daily basis,
173 after which the sampling interval was changed to weekly. A portable automatic water sampler
174 (Bühler 2000, HACH) was used to collect the ozonated effluent and all samples were cooled at
175 7°C before analysis. In addition to automatic water sampling of the ozonated effluent, samples
176 were also taken manually from the effluent of the reference reactor and influent_{bio}. Each
177 wastewater sample was transferred to the lab using amber glass bottles and filtered through
178 glass fibre filters (Macherey-Nagel GF – 3; 0.6 µm) to remove insoluble particles that could
179 interfere with the analysis or damage the analytical equipment.

180 **2.4 Analytical methods**

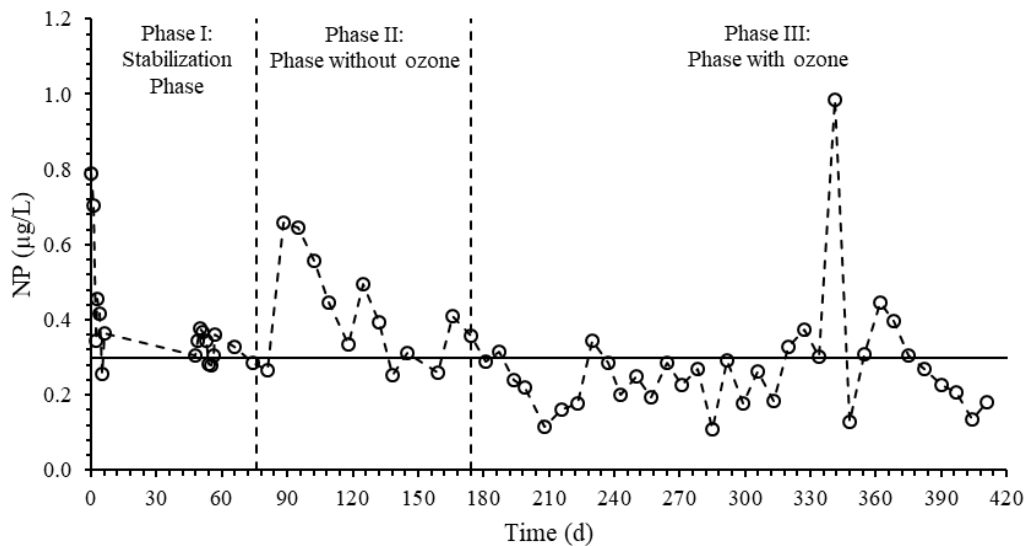
181 The concentration of NP was determined using an HPLC Infinity 1200 LC system (Agilent
182 Technologies, Waldbronn, Germany) coupled to a triple quadrupole mass spectrometer (API
183 3000, Applied Biosystems, Carlsbad, CA, USA), equipped with an electrospray probe working
184 in negative mode. The measurements were performed on a Poroshell EC-C18 (150 x 2.1 mm,
185 2.7 µm) column (Agilent Technologies). The column was operated under isocratic conditions,
186 using a mobile phase consisting of MeOH + 0.05% ammonia:H₂O 90/10 (v/v) (Fisher) at a flow
187 rate of 0.2 mL/min. The sample injection volume was 5 µL, and the column temperature was
188 kept constant at 40°C using a thermostatted column compartment. MS/MS analyses were
189 carried in Multiple Reaction Monitoring (MRM) mode for better sensitivity and specificity.
190 More details on the MS parameters are described in Mignot et al. (2019). Isotopically labelled
191 NP was used as an internal standard for the quantification of NP. Due to the low NP
192 concentrations present in the wastewater samples, a sample pre-treatment was necessary.
193 Liquid-liquid extraction was performed to extract NP from 30 mL of wastewater to 100 µL
194 octanol, as described by Mignot et al. (2019).

195 The sCOD was measured following ISO 6060:1989 (LCK 514 test tubes, Hach). A sample
196 volume of 2 mL was added to a test tube followed by incubation for 15 min at 170 °C. The
197 sCOD was determined by a photometric analysis of the test tube using a DR 3900
198 spectrophotometer (Hach). The Total Organic Carbon (TOC) concentration was measured
199 using a TOC-L analyzer (Shimadzu). A sample volume of at least 15 mL was required for TOC
200 determination. Some of the water samples were also analyzed for ecotoxicity, using
201 commercially available acute ecotoxicity test kits. The 48 h Daphnia Magna water flea
202 immobilization test (OECD, 2004) was used to determine the ecotoxicity based on Daphnia

203 immobilization. In addition, the algae growth inhibition was determined by the 72 h
204 *Pseudokirchneriella subcapitata* algal growth inhibition test (OECD, 2011).

205 3. Results and discussion

206 The pilot plant was operated in different phases. First, a stabilization phase (Phase I) was
207 executed to acclimatize the AS. During Phase II, the ozone step was discontinued for SBR2 as
208 well, and both reactors continued the acclimation. In Phase III, ozonation was resumed until the
209 end of the pilot plant experiment. The NP concentration, sCOD and TOC of the ozonated
210 effluent during the different phases are shown in Fig. 2 and Fig. 3, respectively. The vertical
211 dotted lines indicate each different phase. The horizontal line in Fig. 2 defines the legal limit
212 for NP concentration in effluent water ($0.3 \mu\text{g/L}$) while the sCOD discharge limit (10 days
213 average) is $300 \text{ mg O}_2/\text{L}$ as shown in Fig. 3.



214

215 **Fig. 2.** Nonylphenol concentration of the effluent treated with ozone (○) for the complete duration of the pilot
216 plant experiment.

217

218 3.1 Stabilization phase

219 Phase I, which lasted 76 days, is a stabilization phase. During this phase, the AS of both
220 biological SBR systems was acclimatized to the conditions present in the SBR reactors. The
221 NP concentration of the ozonated effluent was on average $0.38 \mu\text{g/L}$, which is just above the
222 discharge limit of NP of $0.3 \mu\text{g/L}$. As shown in Fig. 3, the average sCOD value of the ozonated
223 effluent during the stabilization period was $152 \text{ mg O}_2/\text{L}$. Fig. 3 also shows the TOC of the
224 ozonated effluent.

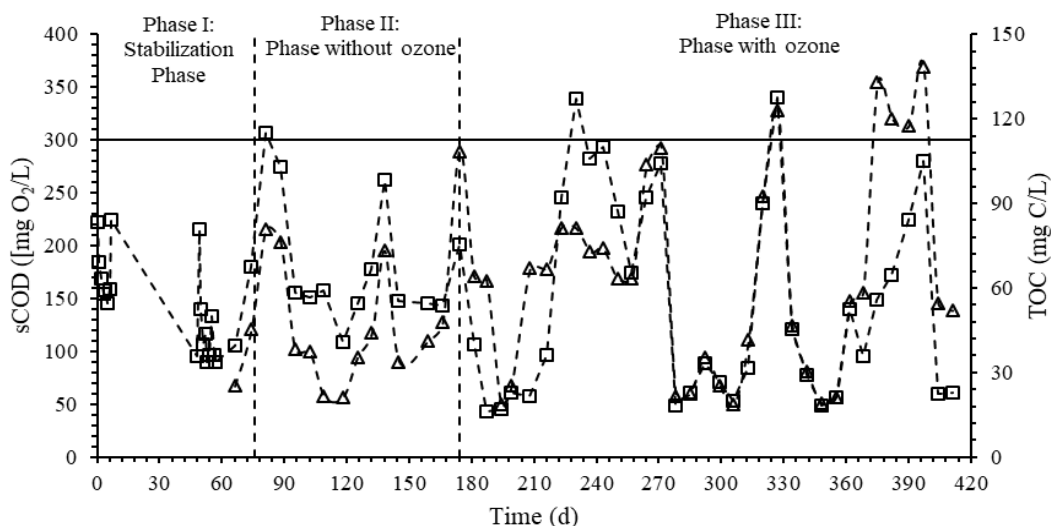


Fig. 3. sCOD (\square) and TOC (Δ) of the effluent treated with ozone for the complete duration of the pilot plant experiment.

3.2 Phase without ozone

During the second phase (total duration of 100 days), the ozone generator was switched off to study the absence of ozonation on the effluent. The NP concentration in the effluent increased from 0.27 $\mu\text{g/L}$ (day 81) to 0.66 $\mu\text{g/L}$ (day 88). A similar trend was observed for the sCOD (maximum 307 $\text{mg O}_2/\text{L}$) and TOC (maximum 81 mg C/L). The NP concentration on day 118 was 0.34 $\mu\text{g/L}$ and increased to 0.50 $\mu\text{g/L}$, reaching a new peak. Even though no ozone was injected, the NP concentration decreased, and the discharge limit of NP was still achieved on days 138 and 159, while an increase in sCOD and TOC from day 118 to 138 is shown in Fig. 3. A peak value in sCOD and TOC of 262 $\text{mg O}_2/\text{L}$ and 73 mg C/L , respectively, occurred on day 138. The average NP concentration, sCOD and TOC during Phase II were 0.41 $\mu\text{g/L}$, 183 $\text{mg O}_2/\text{L}$ and 51 mg C/L , respectively. Compared to the stabilization phase, the average NP and sCOD showed an increase of 7% and 20%, respectively. In general, it can be concluded that the absence of ozonation resulted in a lower NP and sCOD removal. Moreover, peaks in NP and sCOD were observed during Phase II, indicating that the effluent is more prone to fluctuations. These fluctuations in NP, sCOD and TOC in the effluent are partly due to the different tank cleaning activities, meaning that the wastewater composition depends on the content of the tanks that have been cleaned at that time.

3.3 Phase with ozone

At the start of Phase III, the ozone generator was put into operation for SBR 2. During this phase, water samples of the effluent of the reference reactor and the influent_{bio} were analyzed to determine the NP concentration to understand its effect on the obtained effluent. As a result,

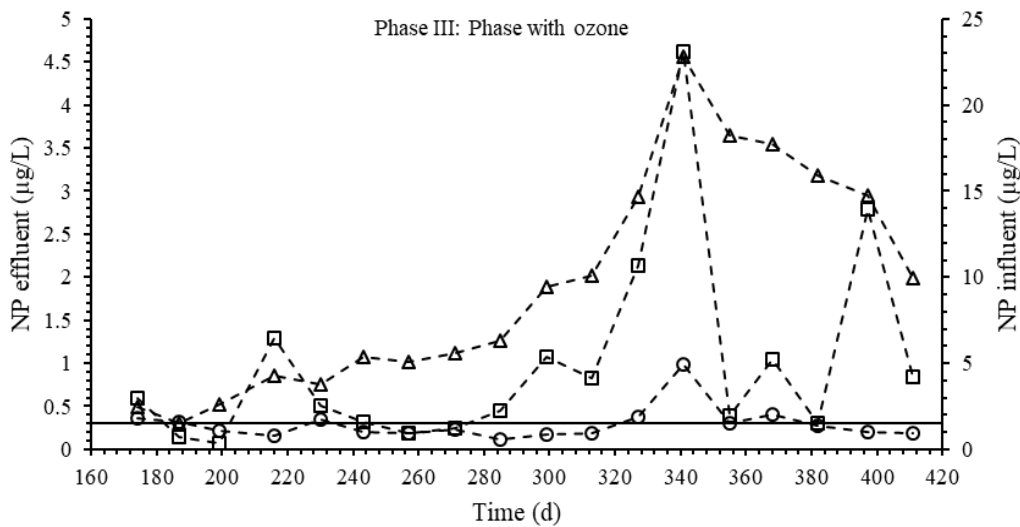
250 a mass balance of NP could be developed. An example is given in Table 1 for day 285 during
 251 phase III.

252 **Table 1.** Nonylphenol, sCOD and TOC of influent_{bio}, SBR 1 and SBR 2 on day 285 (Phase III).

	NP ($\mu\text{g/L}$)	sCOD ($\text{mg O}_2/\text{L}$)	TOC (mg C/L)
Influent _{bio}	2.21	1,950	640
SBR 1	1.27	236	74
SBR 2 + O ₃	0.11	61	22

253

254 In Table 1, the effect of ozonation on the NP, sCOD and TOC removal, compared to influent_{bio},
 255 is presented. The NP removal of SBR 1 is 43% while the ozonated effluent achieved an NP
 256 removal of 95% complying with the discharge limit of 0.3 $\mu\text{g/L}$. This indicates that ozonation
 257 is efficient to ensure the removal of NP from the wastewater to meet the imposed legislation.
 258 For day 285, SBR 1 obtained an sCOD and TOC removal of 88% and 89%, respectively.
 259 Whereas, SBR 2 achieved a higher sCOD and TOC removal of 97% for each, indicating the
 260 positive effect of ozonation.



261

262 **Fig. 4.** Evolution of nonylphenol concentration of the effluent without ozone (Δ), with ozone (\circ) and the
 263 influent (\square).

264 An overall increase in NP concentration in the influent_{bio} was observed in the course of the pilot
 265 plant experiment, reaching a maximum NP concentration of 23.13 $\mu\text{g/L}$ on day 341 before
 266 initiating a decrease as seen in Fig. 4. The NP concentration of the effluents of SBR 1 and 2
 267 also increased during the same period, reaching a maximum of 4.56 and 1 $\mu\text{g/L}$, respectively.
 268 In contrast, the sCOD (Fig. 5) and TOC (Fig. 6) of influent_{bio} remained reasonably constant
 269 during Phase III with an average sCOD and TOC of 2027 $\text{mg O}_2/\text{L}$ and 691 mg C/L ,
 270 respectively.

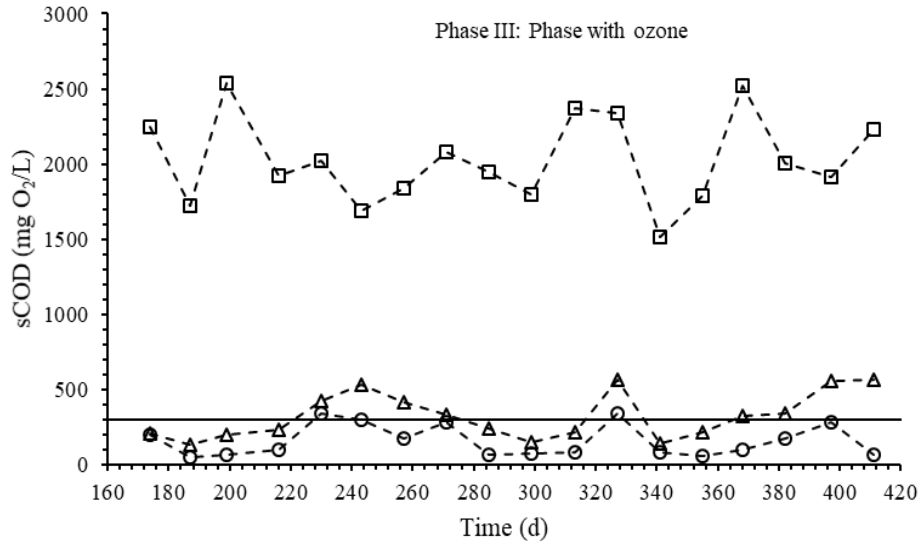


Fig. 5. Evolution of sCOD of the effluent without ozone (Δ), with ozone (\circ) and the influent (\square).

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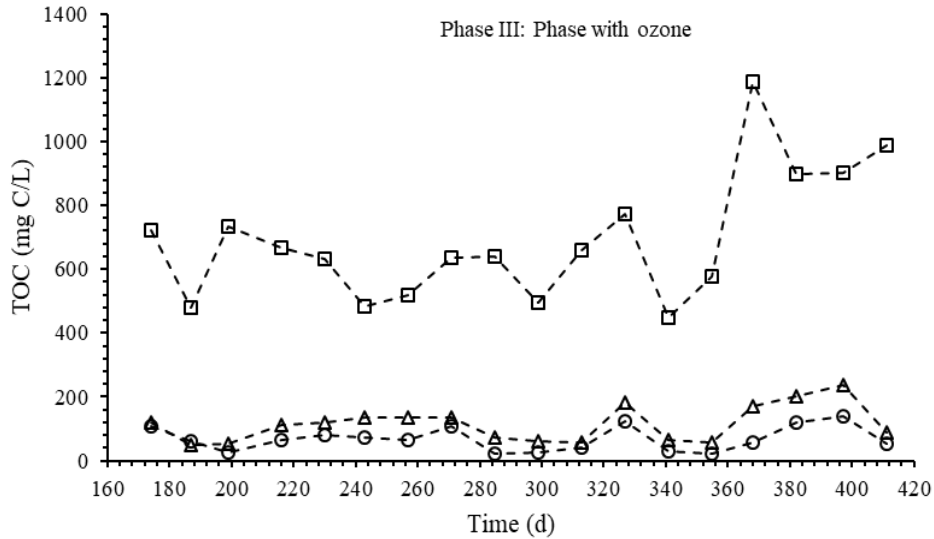
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At the beginning of Phase III, the NP concentration of the ozonated effluent immediately decreased fairly below the imposed discharge limit and in some cases even below the limit of quantitation (LOQ) of the LC-MS analysis ($0.17 \mu\text{g/L}$), as seen in Fig. 4. Likewise, the sCOD and TOC values decreased in the same way during that phase. However, in Fig. 2, it is apparent that the NP concentration was already decreasing at the end of phase II. It is difficult to explain this decrease, but it might be related to a decreasing NP concentration in the influent_{bio}. The NP data of influent_{bio} at the start of phase III was at a low level ($3.00 \mu\text{g/L}$), which appears to support the assumption that the NP loading in the influent_{bio} was rather low at the end of phase II. Until day 313, some fluctuations in the NP concentration of the ozonated effluent are observed (Fig. 4) without exceeding the discharge limit of NP. From day 313, an increase in NP concentration was observed, resulting in a maximum of $0.99 \mu\text{g/L}$ on day 341. The settleability of the AS was poor during this phase, which resulted in a high suspended solids concentration in the effluent. It is known that NP strongly adsorbs to sludge flocs, which could explain the NP peak (Soares et al., 2008). Also, influent_{bio} reached its highest NP concentration in this period. After 4 weeks, the discharge limit of NP was again achieved and maintained until the end of the pilot plant experiment. During phase III, the average NP concentration of the ozonated effluent was $0.29 \mu\text{g/L}$. Furthermore, the imposed discharge limit of NP was, apart from day 320 until 368, always respected. Based on the obtained NP concentrations, it can be concluded that ozone has a positive effect on degrading NP.



292
293 **Fig. 6.** Evolution of TOC of the effluent without ozone (Δ), with ozone (\circ) and the influent (\square).

294 A relationship between the effluent of SBR 1 and the influent_{bio} is depicted in Fig. 4. An
295 increase in NP concentration of the influent_{bio} led to a higher NP concentration in the effluent
296 of SBR 1. Moreover, the effluent of SBR 1 was subjected to the fluctuating NP concentrations
297 from influent_{bio}. In contrast, the NP concentration in the ozonated effluent was relatively
298 constant throughout the experimental campaign. The sCOD and TOC of the effluent of each
299 SBR were in line with the influent_{bio}. Sometimes this resulted in sCOD values above 500 mg
300 O₂/L for the effluent of SBR 1. The ozonated effluent complied with and maintained the
301 discharge limit of sCOD even during sCOD peak loads from the influent_{bio}.

302
303 Based on the results shown in Fig. 4, the removal efficiency of NP during Phase III was
304 calculated. Table 2 summarises the NP, sCOD and TOC removal efficiency of SBR 1 and SBR
305 2.

306
307 **Table 2.** Summary nonylphenol, sCOD and TOC removal efficiency of SBR 1 and SBR 2 during Phase III.

	Average concentration		
	NP ($\mu\text{g/L}$)	COD ($\text{mg O}_2/\text{L}$)	TOC (mg C/L)
Influent _{bio}	4.95	2,027	691
SBR 1	1.89	319	115
SBR 2 + O ₃	0.29	156	68
	Average removal efficiency		
SBR 1/Influent	0.62	0.84	0.83
SBR 2 + O ₃ /Influent	0.94	0.92	0.90
Enhanced removal	0.32	0.08	0.07

308

309 The average NP concentration of influent_{bio} (4.95 µg/L) is in line with previous reported
310 literature. However, the NP data of the influent_{bio} must be interpreted with caution because it is
311 difficult to compare with the literature due to the different wastewater environments. Numerous
312 studies have determined the NP concentration in the raw influent of municipal wastewater
313 treatment plants, which was seen to vary from 0.1 up to 96.4 µg/L (Nakada et al., 2006; Soares
314 et al., 2008; Klečka et al., 2010; Camacho-Muñoz et al., 2014). However, these publications
315 analysed the NP concentration of the raw influent of the wastewater treatment plant instead of
316 the influent_{bio}. Additionally, these wastewater samples were taken from municipal instead of
317 industrial wastewater treatment plants and could therefore contain a lower NP loading.

318

319 The effluent of SBR 1 contained an average NP concentration of 1.89 µg/L which is, compared
320 to the literature, a relatively high concentration for a final effluent after a biological treatment.
321 Johnson et al. (2005) summarized the available literature on NP present in the final effluent of
322 municipal wastewater treatment plants. Interestingly, some effluents have a higher NP
323 concentration than the influent. It is known that NP can be produced during the biological
324 treatment due to the biodegradation of NPEO and NP is therefore often found in higher
325 concentrations in the effluent than in the influent (Soares et al., 2008). Camacho et al., for
326 example, obtained NP concentrations between 5.85 and 25.1 µg/L in the final effluent while
327 the influent contained a range of 1.64 - 1.95 µg/L. In other words, the low NP concentrations
328 in the effluent of SBR 1 could be the result of low a NPEO loading in the influent_{bio}.
329 Furthermore, the effluent of SBR 1 achieved a satisfactory average NP removal efficiency of
330 62% by sole biological treatment. However, a wide range of NP removal efficiencies by sewage
331 treatment (9-94%) are reported in the literature depending to the region and type of treatment
332 process employed (Soares et al., 2008).

333

334 Remarkably, the NP concentration of the ozonated effluent always remained low. As a result,
335 the discharge limit of 0.3 µg/L for NP was almost always achieved as previously reported. The
336 average NP removal efficiency of the ozonated effluent compared to the influent_{bio} was 94%
337 while SBR 1 achieved 62%. Consequently, ozonation significantly improved the average NP
338 removal by 32%.

339

340 In other words, the ozone treatment guarantees an effluent in which the NP concentration
341 remains relatively low, even if peak loads are observed in the influent of the biological
342 treatment, while the effluent obtained after treatment without ozone follows the trend of the

343 peak loads of the influent. It can even be concluded that ozone treatment is necessary to achieve
344 the NP discharge limit of 0.3 µg/L.

345

346 A difference in sCOD and TOC values between the two different effluents is also clearly visible
347 in Fig. 5 and Fig. 6. The average sCOD and TOC values of the effluent of SBR 1 were 319 mg
348 O₂/L and 115 mg C/L, respectively. If these results are compared to the sCOD and TOC values
349 of the influent_{bio}, an average removal efficiency for both parameters can be determined. The
350 biological wastewater treatment without an ozone polishing treatment reached an average
351 sCOD and TOC removal efficiency of 84% and 83%, respectively. Furthermore, lower sCOD
352 and TOC values for the ozonated effluent were observed: 155 mg O₂/L and 68 mg C/L, resulting
353 in an even higher (however minor) average sCOD and TOC removal efficiency of 92% and
354 90%, which is positive. Worth noting, during Phase III, the enhanced sCOD removal efficiency
355 was necessary to meet the discharge limit of sCOD. The maximum sCOD load may not be
356 higher than 500 mg O₂/L and the maximum moving 10-day sCOD average is 300 mg O₂/L. The
357 average sCOD of SBR 1 was 319 mg O₂/L compared to 155 mg O₂/L for the ozonated effluent.
358 In other words, ozonation could guarantee an average sCOD lower than 300 mg O₂/L.

359 It is true that ozonation of phenolic compounds such as NP can lead to the formation of toxic
360 by-products. Most literature sources refer to possible degradation products of phenol while
361 addressing the degradation of NP. The decomposition of NP by ozonation can lead to a cleavage
362 of the aromatic ring or the decomposition of the alkyl side chain (ElShafei et al., 2017). Firstly,
363 the ring cleavage results in the formation of different aldehydes and carboxylic acids, e.g.
364 maleic acid (Olmez-Hanci et al., 2013). These compounds are less harmful compared to the
365 phenolic precursor. Secondly, the decomposition of the alkyl side chain can result in the
366 formation of dihydroxy phenols due to the addition of hydroxyl radicals to the ortho or para
367 position. Such phenolic intermediate by-products could be more toxic than NP as stated by
368 reviewer's comment. However, Ning et al. (2007) indicated that the site of the ozone attack was
369 not on the alkyl chain but at the aromatic ring structure.

370 Therefore, some water samples obtained during Phase III were also collected for an ecotoxicity
371 analysis. The results of the ecotoxicity analyses of the reference effluent (SBR 1), ozonated
372 effluent (SBR 2) and the influent_{bio} are shown in Table 3.

373

374 **Table 3.** Ecotoxicity in the ozonated effluent compared to effluent from the reference reactor and the influent_{bio}.

		Growth inhibition (%)	Immobilization (%)
Day 194	SBR 2 + O ₃	60 ± 14	70
	Influent	100 ± 0	100
Day 199	SBR 1	94 ± 10	20
	SBR 2 + O ₃	83 ± 15	55
Day 208	SBR 2 + O ₃	100 ± 0	80

375

376 The results, shown in Table 3, indicate that the influent_{bio} showed complete inhibition and
 377 immobilization. The influent_{bio} is wastewater that only underwent a physicochemical treatment,
 378 meaning that it still has a high pollution degree. In terms of algae inhibition, no significant
 379 difference was observed between the ozonated and reference effluents (same order of
 380 magnitude). Interestingly, the reference effluent showed the lowest Daphnia immobilization,
 381 up to 20%, while this was about 70% for the ozonated effluent. A direct explanation could not
 382 be identified. Petala et al. (2006) and Von Gunten (2018) reported that the formation of
 383 degradation products by the ozone polishing step could cause higher ecotoxicity. Furthermore,
 384 Poelmans et al. (2020) investigated the effect of ozonation as polishing step on the removal of
 385 ecotoxicity from tank truck cleaning wastewater. Despite its positive effect on the effluent
 386 quality with a COD-decrease up to 70%, the ecotoxicity is highly dependent on the formation
 387 of ecotoxic by-products which may even lead to an increase in ecotoxicity. In addition,
 388 Poelmans et al. (2020) stated that ozonation as a pre-treatment step for wastewater from tank
 389 truck cleaning did not create a higher ecological effluent quality. This paper therefore confirms
 390 the importance of the effect of ozonation on ecotoxicity.

391

392 **4. Conclusions**

393 The effect of ozone as a polishing treatment was studied by using a pilot-scale biological SBR
 394 system combined with an ozonation unit. During the ozonation phase, the obtained effluent
 395 from the SBR underwent an ozonation treatment as a polishing step. The average nonylphenol
 396 concentration, sCOD and TOC values during phase III were 0.29 µg/L, 155 mg O₂/L and 68
 397 mg C/L, respectively. The combination of biological wastewater treatment with ozonation as a
 398 polishing step was able to remove on average 94% of the nonylphenol from the influent_{bio},
 399 while SBR 1 could only remove 62%. In other words, ozonation significantly improved the
 400 average nonylphenol removal efficiency by approximately 32%.

401 Moreover, the ozonated effluent was less influenced by the nonylphenol peak concentration
402 observed in the influent_{bio} compared to the effluent of the reference SBR. These findings
403 suggest that the ozone treatment guarantees an effluent in which the nonylphenol concentration
404 remains relatively low, even if peak loads are observed in the influent of biological treatment.
405 At the same time, the effluent without ozone follows the trend of the peak loads in the
406 influent_{bio}. Worth noting, the sCOD and TOC removal were also slightly enhanced by
407 introducing ozone as a post-treatment after the biology.

408 In general, it can be concluded that ozone treatment is efficient and necessary to achieve the
409 discharge limit of 0.3 µg/L for nonylphenol in the effluent. The optimization of the ozonation
410 process was proved efficient but could be further explored in future research. It might be
411 interesting to explore different process parameters of the ozonation polishing step, such as the
412 residence time ozone reactor or the ozone concentration as well as to study different industrial
413 wastewater matrices.

414

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419

420 **References**

421 Acir, I. H., & Guenther, K. (2018). Endocrine-disrupting metabolites of alkylphenol
422 ethoxylates—A critical review of analytical methods, environmental occurrences, toxicity, and
423 regulation. *Science of the Total Environment*, 635, 1530-1546.

424

425 Ahel, M., Giger, W., & Koch, M. (1994). Behaviour of alkylphenol polyethoxylate surfactants
426 in the aquatic environment—I. Occurrence and transformation in sewage treatment. *Water*
427 *Research*, 28(5), 1131-1142.

428

429 Amaro, A. A., Esposito, A. I., Mirisola, V., Mehilli, A., Rosano, C., Noonan, D. M., ... &
430 Angelini, G. (2014). Endocrine disruptor agent nonyl phenol exerts an estrogen-like
431 transcriptional activity on estrogen receptor positive breast cancer cells. *Current medicinal*
432 *chemistry*, 21(5), 630-640.

433

434 AMEC. European Chemicals Agency. (2012). Abatement Costs of Certain Hazardous
435 Chemicals. Lot 2 (Nonylphenol and nonylphenol ethoxylates in textiles). *AMEC Environmental*
436 *& Infrastructure UK Limited*.

437

438 Araujo, F. G., Bauerfeldt, G. F., & Cid, Y. P. (2018). Nonylphenol: Properties, legislation,
439 toxicity and determination. *Anais da Academia Brasileira de Ciências*, 90(2), 1903-1918.

440

441 Bontje, D., Hermens, J., Vermeire, T., & Damstra, T. (2004). Integrated Risk Assessment:
442 Nonylphenol Case Study. Report prepared for the WHO/UNEP/ILO International Programme
443 on Chemical Safety. *World Health Organization (WHO), United Nations Environment*
444 *Programme (UNEP) & International Labour Organization (ILO)*.

445

446 Camacho-Muñoz, D., Martín, J., Santos, J. L., Aparicio, I., & Alonso, E. (2014). Occurrence of
447 surfactants in wastewater: hourly and seasonal variations in urban and industrial wastewaters
448 from Seville (Southern Spain). *Science of the total environment*, 468, 977-984.

449

450 Cornelis, G., Boussu, K., Van der Bruggen, B., Devreese, I., & Vandecasteele, C. (2005).
451 Nanofiltration of nonionic surfactants: effect of the molecular weight cutoff and contact angle
452 on flux behavior. *Industrial & engineering chemistry research*, 44(20), 7652-7658.

453

454 Dewil, R., Mantzavinos, D., Poulios, I., & Rodrigo, M. A. (2017). New perspectives for
455 advanced oxidation processes. *Journal of environmental management*, 195, 93-99.

456

457 ECB, European Chemicals Bureau, 2002. European Union Risk Assessment Report 4-
458 Nonylphenol (branched) and Nonylphenol. 2nd Priority List, Volume 10. *European Chemicals*
459 *Bureau, European Commission*, (EUR 20387 EN).

460

461 ElShafei, G. M. S., Yehia, F. Z., Eshaq, G., & ElMetwally, A. E. (2017). Enhanced degradation
462 of nonylphenol at neutral pH by ultrasonic assisted-heterogeneous Fenton using nano zero
463 valent metals. *Separation and Purification Technology*, 178, 122-129.

464

465 European Council Directive 2003/53/EC of 18 June 2003 amending for the 26th time Council
466 Directive 76/769/EEC relating to restrictions on the marketing and use of certain dangerous
467 substances and preparations (nonylphenol, nonylphenol ethoxylate and cement).

468
469 ECHA. European Chemical Agency. (2014). Background document to the Opinion on the
470 Annex XV dossier proposing restrictions on nonylphenol ethoxylate.
471
472 Fujita, M., Ike, M., Mori, K., Kaku, H., Sakaguchi, Y., Asano, M., ... & Nishihara, T. (2000).
473 Behaviour of nonylphenol ethoxylates in sewage treatment plants in Japan—biotransformation
474 and ecotoxicity. *Water Science and Technology*, 42(7-8), 23-30.
475
476 Gabriel, F. L., Routledge, E. J., Heidelberger, A., Rentsch, D., Guenther, K., Giger, W., ... &
477 Kohler, H. P. E. (2008). Isomer-specific degradation and endocrine disrupting activity of
478 nonylphenols. *Environmental science & technology*, 42(17), 6399-6408.
479
480 Giger, W., Brunner, P. H., & Schaffner, C. (1984). 4-Nonylphenol in sewage sludge:
481 accumulation of toxic metabolites from nonionic surfactants. *Science*, 225(4662), 623-625.
482
483 Glaze, W. H., Kang, J. W., & Chapin, D. H. (1987). The chemistry of water treatment processes
484 involving ozone, hydrogen peroxide and ultraviolet radiation.
485
486 Gottschalk, C., Libra, J. A., & Saupe, A. (2009). Ozonation of water and wastewater: A
487 practical guide to understanding ozone and its applications. John Wiley & Sons.
488
489 In, S. J., Kim, S. H., Go, R. E., Hwang, K. A., & Choi, K. C. (2015). Benzophenone-1 and
490 nonylphenol stimulated MCF-7 breast cancer growth by regulating cell cycle and metastasis-
491 related genes via an estrogen receptor α -dependent pathway. *Journal of Toxicology and*
492 *Environmental Health, Part A*, 78(8), 492-505.
493
494 Johnson, A. C., Aerni, H. R., Gerritsen, A., Gibert, M., Giger, W., Hylland, K., ... & Wettstein,
495 F. E. (2005). Comparing steroid estrogen, and nonylphenol content across a range of European
496 sewage plants with different treatment and management practices. *Water research*, 39(1), 47-
497 58.
498
499 Kim, J., Korshin, G. V., & Velichenko, A. B. (2005). Comparative study of electrochemical
500 degradation and ozonation of nonylphenol. *Water research*, 39(12), 2527-2534.
501

502 Klečka, G. M., Naylor, C. G., Staples, C. A., & Losey, B. (2010). Occurrence of nonylphenol
503 ethoxylates and their metabolites in municipal wastewater treatment plants and receiving
504 waters. *Water environment research*, 82(5), 447-454.

505

506 Kovarova, J., Blahova, J., Divisova, L., & Svobodova, Z. (2013). Alkylphenol ethoxylates and
507 alkylphenols—update information on occurrence, fate and toxicity in aquatic
508 environment. *Polish Journal of Veterinary Sciences*, 16(4).

509

510 Mao, Z., Zheng, X. F., Zhang, Y. Q., Tao, X. X., Li, Y., & Wang, W. (2012). Occurrence and
511 biodegradation of nonylphenol in the environment. *International journal of molecular
512 sciences*, 13(1), 491-505.

513

514 Mignot, M., Nagels, M., Poelmans, S., Kensert, A., Dries, J., Dewi, R., & Cabooter, D. (2019).
515 Fast liquid chromatography-tandem mass spectrometry methodology for the analysis of
516 alkylphenols and their ethoxylates in wastewater samples from the tank truck cleaning
517 industry. *Analytical and bioanalytical chemistry*, 411(8), 1611-1621.

518

519 Nakada, N., Tanishima, T., Shinohara, H., Kiri, K., & Takada, H. (2006). Pharmaceutical
520 chemicals and endocrine disrupters in municipal wastewater in Tokyo and their removal during
521 activated sludge treatment. *Water research*, 40(17), 3297-3303.

522

523 Ning, B., Graham, N. J., & Zhang, Y. (2007). Degradation of octylphenol and nonylphenol by
524 ozone—Part I: Direct reaction. *Chemosphere*, 68(6), 1163-1172.

525

526 Ning, B., Graham, N. J., & Zhang, Y. (2007). Degradation of octylphenol and nonylphenol by
527 ozone—Part II: Indirect reaction. *Chemosphere*, 68(6), 1173-1179.

528

529 Noorimotlagh, Z., Mirzaee, S. A., Martinez, S. S., Rachoń, D., Hoseinzadeh, M., & Jaafarzadeh,
530 N. (2020). Environmental exposure to nonylphenol and cancer progression Risk—A systematic
531 review. *Environmental Research*, 109263.

532

533 OECD. (2011). Test No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test.

534

535 Oller, I., Malato, S., & Sánchez-Pérez, J. (2011). Combination of advanced oxidation processes
536 and biological treatments for wastewater decontamination—a review. *Science of the total*
537 *environment*, 409(20), 4141-4166.

538

539 Olmez-Hanci, T., Arslan-Alaton, I., & Basar, G. (2011). Multivariate analysis of anionic,
540 cationic and nonionic textile surfactant degradation with the H₂O₂/UV-C process by using the
541 capabilities of response surface methodology. *Journal of hazardous materials*, 185(1), 193-
542 203.

543

544 Olmez-Hanci, T., & Arslan-Alaton, I. (2013). Comparison of sulfate and hydroxyl radical based
545 advanced oxidation of phenol. *Chemical Engineering Journal*, 224, 10-16.

546

547 Patoczka, J., & Pulliam, G. W. (1990). Biodegradation and secondary effluent toxicity of
548 ethoxylated surfactants. *Water Research*, 24(8), 965-972.

549

550 Petala, M., Samaras, P., Zouboulis, A., Kungolos, A., & Sakellariopoulos, G. (2006).
551 Ecotoxicological properties of wastewater treated using tertiary methods. *Environmental*
552 *Toxicology: An International Journal*, 21(4), 417-424.

553

554 Poelmans, S., Nagels, M., Mignot, M., Dewil, R., Cabooter, D., & Dries, J. (2020). Effect of
555 ozonation as pre-treatment and polishing step on removal of ecotoxicity and alkylphenol
556 ethoxylates from tank truck cleaning wastewater. *Journal of Water Process Engineering*, 37,
557 101441.

558

559 Renner, R. (1997). European bans on surfactant trigger transatlantic debate. *Environmental*
560 *Science & Technology*, 31(7), 316A-320A.

561

562 Servos, M. R. (1999). Review of the aquatic toxicity, estrogenic responses and bioaccumulation
563 of alkylphenols and alkylphenol polyethoxylates. *Water Quality Research Journal*, 34(1), 123-
564 178.

565

566 Sharma, V. K., Anquandah, G. A., Yngard, R. A., Kim, H., Fekete, J., Bouzek, K., ... &
567 Golovko, D. (2009). Nonylphenol, octylphenol, and bisphenol-A in the aquatic environment: a

568 review on occurrence, fate, and treatment. *Journal of Environmental Science and Health Part*
569 *A*, 44(5), 423-442.

570

571 Soares, A., Guieysse, B., Jefferson, B., Cartmell, E., & Lester, J. N. (2008). Nonylphenol in the
572 environment: a critical review on occurrence, fate, toxicity and treatment in
573 wastewaters. *Environment International*, 34(7), 1033-1049.

574

575 Soto, A. M., Justicia, H., Wray, J. W., & Sonnenschein, C. (1991). p-Nonyl-phenol: an
576 estrogenic xenobiotic released from "modified" polystyrene. *Environmental health*
577 *perspectives*, 92, 167-173.

578

579 Suslow, T. V. (2004). Oxidation-reduction potential (ORP) for water disinfection monitoring,
580 control, and documentation.

581

582 Talmage, S. S. (1994). Environmental and human safety of major surfactants: alcohol
583 ethoxylates and alkylphenol ethoxylates. CRC Press.

584

585 Van Aken, P., Van den Broeck, R., Degève, J., & Dewil, R. (2015). The effect of ozonation
586 on the toxicity and biodegradability of 2, 4-dichlorophenol-containing wastewater. *Chemical*
587 *Engineering Journal*, 280, 728-736.

588

589 Van Aken, P., Van den Broeck, R., Degève, J., & Dewil, R. (2017). A pilot-scale coupling of
590 ozonation and biodegradation of 2, 4-dichlorophenol-containing wastewater: The effect of
591 biomass acclimation towards chlorophenol and intermediate ozonation products. *Journal Of*
592 *Cleaner Production*, 161, 1432-1441.

593

594 von Gunten, U. (2018). Oxidation processes in water treatment: are we on
595 track?. *Environmental science & technology*, 52(9), 5062-5075.

596

597 Yang, O., Kim, H. L., Weon, J. I., & Seo, Y. R. (2015). Endocrine-disrupting chemicals: review
598 of toxicological mechanisms using molecular pathway analysis. *Journal of cancer*
599 *prevention*, 20(1), 12.