

1 **Best available technologies and treatment trains to address current challenges in urban**
2 **wastewater reuse for irrigation of crops in EU countries**

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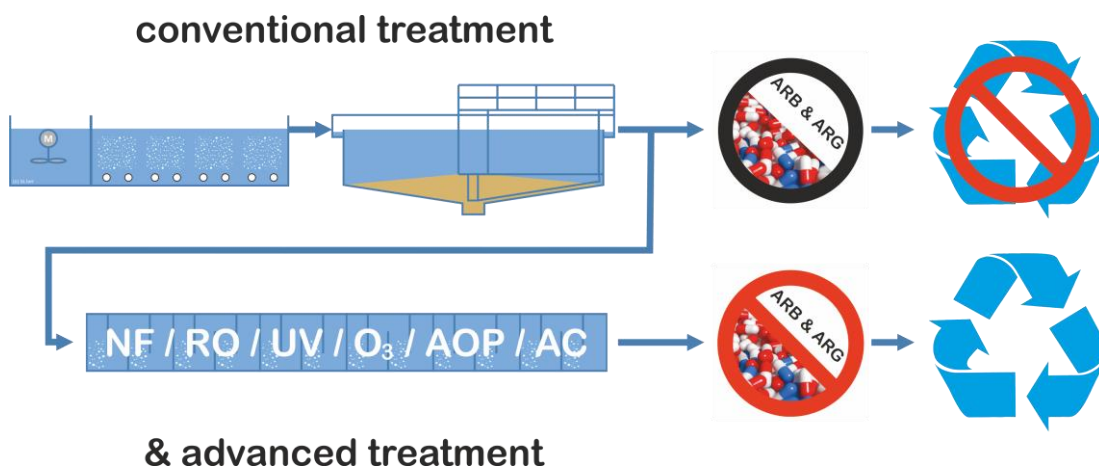
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30 **Highlights**

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- 31 • This work gathers the efforts of international experts from NEREUS COST
32 Action
- 33 • Advantages and drawbacks of BATs discussed according to CECs removal and
34 AR control
- 35 • Possible advanced treatment options to make wastewater reuse safer
36 recommended
- 37 • Smart combination of BATs and a suitable monitoring program necessary for a
38 safe reuse
- 39 • Further comparative studies among different advanced treatment methods
40 recommended

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43 **Abstract**

44 Conventional urban wastewater treatment plants (UWTPs) are poorly effective in the removal
45 of most contaminants of emerging concern (CECs), including antibiotics, antibiotic resistant
46 bacteria and antibiotic resistance genes (ARB&ARGs). These contaminants result in some
47 concern for the environment and human health, in particular if UWTPs effluents are reused for
48 crop irrigation. Recently, stakeholders' interest further increased in Europe, because the
49 European Commission is currently developing a regulation on water reuse. Likely, conventional
50 UWTPs will require additional advanced treatment steps to meet water quality limits yet to be
51 officially established for wastewater reuse. Even though it seems that CECs will not be included
52 in the proposed regulation, the aim of this paper is to provide a technical contribution to this
53 discussion as well as to support stakeholders by recommending possible advanced treatment
54 options, in particular with regard to the removal of CECs and ARB&ARGs. Taking into account
55 the current knowledge and the precautionary principle, any new or revised water-related
56 Directive should address such contaminants. Hence, this review paper gathers the efforts of a
57 group of international experts, members of the NEREUS COST Action ES1403, who for three
58 years have been constructively discussing the efficiency of the best available technologies
59 (BATs) for urban wastewater treatment to abate CECs and ARB&ARGs. In particular,
60 ozonation, activated carbon adsorption, chemical disinfectants, UV radiation, advanced
61 oxidation processes (AOPs) and membrane filtration are discussed with regard to their
62 capability to effectively remove CECs and ARB&ARGs, as well as their advantages and
63 drawbacks. Moreover, a comparison among the above-mentioned processes is performed for

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64 CECs relevant for crop uptake. Finally, possible treatment trains including the above-discussed
65 BATs are discussed, issuing end-use specific recommendations which will be useful to UWTPs
66 managers to select the most suitable options to be implemented at their own facilities to
67 successfully address wastewater reuse challenges.

68

69 Keywords: activated carbon, advanced oxidation processes, antibiotic resistance,
70 contaminants of emerging concern, disinfection, ozonation

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- 72 List of abbreviations
- 73 ARB= antibiotic resistant bacteria
- 74 ARGs= antibiotic resistance genes
- 75 AOPs= advanced oxidation processes
- 76 BAC= biological activated carbon
- 77 CBZ= carbamazepine
- 78 CECs= contaminants of emerging concern
- 79 CPC= compound parabolic collector
- 80 DBPs= disinfection by products
- 81 DCF= diclofenac
- 82 DOC= dissolved organic carbon
- 83 ERY= erythromycin
- 84 FRC= free residual chlorine
- 85 GAC= granular activated carbon
- 86 HO^{*}= hydroxyl radical
- 87 LRV= Log removal value
- 88 MDR= multi drug resistant
- 89 MF= microfiltration
- 90 NDMA= N-nitrosodimethylamine
- 91 NF= nanofiltration
- 92 PAC= powdered activated carbon

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- 93 RO= reverse osmosis
- 94 TMP= transmembrane pressure
- 95 UF= ultrafiltration
- 96 SMX= sulfamethoxazole
- 97 UWTPs = urban wastewater treatment plants

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98 **1. Introduction**

99 Wastewater reuse is one of the most important alternatives to conventional water sources to
100 address water scarcity. As a matter of fact, around 1.2 billion people live in areas affected by
101 serious water scarcity conditions (United Nations, 2014) and 1.8 billion people are expected to
102 be living in countries or regions affected by water scarcity by 2025, according to United Nations
103 reports (United Nations, 2014; FAO, 2014). Wastewater reuse for irrigation in agriculture is by
104 far the most established end-use for reclaimed water (Dreschel et al., 2010a), in low-income
105 countries as well as in arid and semi-arid ones (Dreschel et al., 2010b). However, whilst solving
106 water scarcity, wastewater reuse can generate public health risks if treatment, storage and piping
107 are not adequate. The main risk, in particular in low-income countries, is related to consumption
108 of raw or undercooked vegetables contaminated with pathogenic microorganisms stemming
109 from the use of untreated or poorly treated wastewater for crop irrigation (Fuhrmann et al.,
110 2016). In countries of higher income level, wastewater reuse for irrigation is regulated, at least
111 in some of them (Paranychianakis et al., 2015), and concerns tend to shift from microbial risk
112 (effective disinfection processes are typically included in the treatment train) to contaminants
113 of emerging concern (CECs), such as pesticides, pharmaceuticals, illicit drugs, synthetic and
114 natural hormones, personal care products, and resistant microorganisms (i.e. antibiotic resistant
115 bacteria and genes (ARB&ARGs)). However, neither the release of CECs from urban
116 wastewater treatment plants (UWTPs) into the environment (except for Switzerland) nor their
117 occurrence in wastewater for agricultural reuse has been regulated so far. CECs monitoring in
118 UWTPs effluents to reuse for crop irrigation is one of the main debated issues among scientists,

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119 policy makers and stakeholders at EU level (Christou et al., 2017a, Piña et al., 2018, Rizzo et
120 al., 2018; Deng et al., 2019) even in relation to the regulation for wastewater reuse which is
121 about to be approved by the Parliament (European Parliament, 2019).

122

123 According to scientific literature, conventional treatment trains in UWTs are poorly effective
124 to comprehensively remove CECs (Petrie et al., 2015; Falas et al., 2016; Krzeminski et al.,
125 2019), which can finally be released into the environment, constituting a particular concern
126 when effluents are reused for crop irrigation. To be able to meet stringent limits for wastewater
127 reuse as well as to effectively remove CECs, advanced treatment steps should be implemented
128 in conventional UWTs (Krzeminski et al., 2019; Rizzo et al., 2019a). However, while the
129 effect of biological processes (Boshir Ahmed et al., 2017; Tiwari et al., 2017; Krzeminski et
130 al., 2019) and advanced treatment technologies (Miklos et al., 2018; von Gunten, 2018;
131 Roccaro, 2018; Marron et al., 2019; Rizzo et al., 2019a; Siegrist et al. 2019) on chemical CECs
132 has been reviewed in different papers, less information is available about ARB&ARGs and,
133 most importantly, on possible treatment trains combining several processes to successfully
134 address these challenges.

135

136 This review paper gathers the efforts of a group of international experts, members of the
137 NEREUS COST Action ES1403¹ “New and emerging challenges and opportunities in

¹ COST Action ES1403 New and emerging challenges and opportunities in wastewater reuse (NEREUS),
<http://www.nereus-cost.eu>.

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138 wastewater reuse” (Fatta-Kassinos et al., 2015), who for three years have been constructively
139 discussing the effect of the best available technologies (BATs) for urban wastewater treatment
140 on CECs and ARB&ARGs. Accordingly, the objective of this paper is to introduce and discuss
141 the BATs for advanced treatment of urban wastewater, as well as possible treatment trains to
142 control the release of CECs, including ARB&ARGs, to produce wastewater for safe and
143 sustainable reuse practices in agriculture. In particular, the capability of ozonation, activated
144 carbon adsorption, chemical oxidants/disinfectants, UV radiation, advanced oxidation
145 processes (AOPs) and membrane filtration to abate CECs and ARB&ARGs are discussed
146 including the advantages and drawbacks of these processes. Moreover, a comparison among
147 the above-mentioned processes is performed for CECs relevant for crop uptake. It is noteworthy
148 that only results from investigations at pilot or full-scale on real wastewater were considered.
149 Subsequently, possible treatment trains including the above-discussed BATs are presented and
150 recommended for possible application in the EU and other developed countries. Finally,
151 possible advantages, drawbacks and recommendations of the proposed treatment trains are
152 summarized.

153

154 **2. Overview of the BATs for advanced treatment and reuse of urban wastewater:**

155 **CECs abatement, effect on ARB&ARGs and process drawbacks**

156 The occurrence of CECs into the environment is related to different human activities (Verlicchi
157 et al., 2015; Bilal et al., 2019a, b) and it has been associated to biological adverse effects on
158 living organisms such as toxicity, endocrine disruption and antibiotic resistance in

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159 microorganisms (Manaia, 2017; López-Pacheco et al., 2019; Ma et al., 2019). Specifically,
160 several CECs have been found to increase the risks for human-health, because they finally cause
161 imbalance to hormonal and male/female reproductive systems and different disorders, namely
162 metabolism, neurological, and immunological ones (López-Pacheco et al., 2019; Pedrazzani et
163 al., 2019; Rueda-Ruzafa et al., 2019).

164 In 2015, the European Commission established the EU Watch List (Decision 2015/495/EU) to
165 monitor 17 CECs in water. The target CECs belong to different categories including antibiotics,
166 estrogenic hormones, non-steroidal anti-inflammatory compounds, pesticides and herbicides,
167 UV filters, and they were selected according to their potential to cause damage to aquatic
168 environments and to pose a significant risk at European Union level, but for which monitoring
169 data are insufficient to come to a conclusion regarding the actual posed risk.

170 UWTPs are recognized among the main anthropogenic sources for the release of CECs and
171 ARB&ARGs into the environment, therefore, taking into account the environment and human
172 health concerns related to their occurrence in UWTPs effluents and into the environment,
173 different advanced treatment technologies have been investigated so far to find effective
174 solutions to minimize their release. In the following sub-paragraphs, the BATs for advanced
175 treatment of urban wastewater are introduced to evaluate their effect on CECs and
176 ARB&ARGs. Possible advantages and drawbacks of these processes are also discussed
177 according to the relevant scientific literature.

178

179 2.1 Ozonation

180 2.1.1 Abatement of CECs

181 The oxidation capacity of the ozone process relies on the strong oxidation potential of both,
182 molecular ozone and HO radicals (HO[•]) (2.07 and 2.8 V against standard hydrogen electrode,

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183 respectively). While ozone reacts selectively with compounds containing electron-rich moieties
184 (such as olefins, deprotonated amines or activated aromatics), HO[•] exhibit a low selectivity and
185 fast reaction with a wide range of organic and inorganic compounds (von Sonntag, 2007).
186 Ozonation and other oxidation-based processes were originally applied for disinfection
187 purposes in drinking water treatment, but have been widely investigated for the abatement of
188 different CECs from urban wastewater since more than 10 years (Ternes et al., 2003). Based on
189 the reaction rate constants with ozone and HO[•], CEC abatement can be predicted in municipal
190 wastewater (Lee et al. 2013). Hollender et al. (2009) and Bourgin et al. (2018) investigated the
191 abatement of 220-550 micropollutants at two full-scale UWTPs upgraded with ozonation
192 (followed by sand filtration). Compounds such as sulfamethoxazole, diclofenac, or
193 carbamazepine with high apparent second-order rate constants at pH 7 ($k_{O_3, pH7} > 10^3$) were
194 abated by more than 80% at a specific ozone dose of 0.4 g O₃/g dissolved organic carbon
195 (DOC). Compounds more refractory to oxidation by ozone ($k_{O_3, pH7} = 10^2 - 10^3$), such as
196 bezafibrate and benzotriazole, were abated by 80% only at a higher ozone dose (~0.6 g O₃/g
197 DOC). The high efficiency of ozonation in the abatement of CECs from wastewater was also
198 confirmed in other studies on a smaller group of compounds (e.g., Antoniou et al. 2013;
199 Magdeburg et al. 2014). After ozonation, a biological post-treatment (sand filter or biological
200 activated carbon (BAC) filter) is recommended to eliminate possible negative ecotoxicological
201 effects or by-products generated during ozonation (Von Gunten, 2018; Bacaro et al. 2019).

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203 2.1.2 Effect on ARB&ARGs

204 Mechanisms for disinfection or inactivation of bacteria by ozone exposure include the
205 disruption of bacterial cell walls (leading to the release of intracellular constituents), damage of
206 nucleic acids (breaking aromatic structure), and breakage of carbon-nitrogen bonds of proteins
207 leading to depolymerisation (Alexander et al., 2016, Michael-Kordatou et al., 2018). The
208 inactivation efficiency by ozonation depends on the susceptibility of the target organism and
209 ozone exposure, which is a function of the wastewater characteristics and transferred ozone
210 dose. Unlike CECs, the effect of ozonation on ARB&ARGs has not been investigated
211 systematically and thoroughly so far. Alexander et al. (2016) observed diverse patterns of
212 resistances and susceptibilities of opportunistic bacteria and accumulations of some ARGs
213 during ozone treatment (0.9 ± 0.1 g O₃/g DOC) of treated wastewater. Ozone affected
214 microorganisms in different ways, with a high susceptibility of enterococci (almost 99%
215 reduction) compared to *Pseudomonas aeruginosa*, that displayed only minor changes in
216 abundance after treatment. The investigated ARGs demonstrated an even more diverse pattern
217 with 2 orders of magnitude reduction of erythromycin resistance gene (*ermB*) but a
218 simultaneous increase in the abundance of ARGs (*vanA*, *bla_{VIM}*) within the surviving
219 wastewater population. Ozonation operated at high contact time (40 min) with an ozone dose
220 of 0.25 g O₃/g DOC was capable of inactivating total as well as antibiotic (sulfamethoxazole
221 and trimethoprim) resistant *Escherichia coli* (*E. coli*), with the simultaneous reduction of the
222 abundance of the examined genes (Iakovides et al., 2019). Accordingly, the studies published
223 so far confirm that the ozonation process is effective in the inactivation of ARB and to some

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224 extent in the removal of ARGs (Lüddeke et al., 2014; Zhuang et al., 2015; Alexander et al.,
225 2016; Zheng et al., 2017; Sousa et al., 2017), but it seems that the process may also select for
226 bacterial population (Alexander et al., 2016; Sousa et al., 2017; Czekalski et al., 2016).
227 Regrowth of ARB during biological sand-filtration following ozonation was found to partly
228 compensate inactivation during ozonation (Czekalski et al., 2016). Moreover, mobile genetic
229 elements may reach pre-treatment levels after some days of storage (Sousa et al., 2017), which
230 can be of concern for wastewater reuse practice where treated effluents may be stored for some
231 days before use (Iakovides et al., 2019).

232

233 2.1.3 Formation of oxidation by-products

234 Ozonation can result in the formation of biologically potent (e.g. toxic, mutagenic) oxidation
235 by-products. Among them, N-nitrosodimethylamine (NDMA) and bromate are of particular
236 concern for human health because they are potentially carcinogenic. Therefore, NDMA and
237 bromate need to be measured to test the feasibility of ozonation as an option for advanced
238 wastewater treatment at a specific location (Schindler Wildhaber et al., 2015). Only if the
239 concentrations expected after dilution of discharged effluents are clearly below (potential)
240 drinking water standards (10 µg/L for bromate, 10 ng/L for NDMA, Bourgin et al., 2018),
241 ozonation is considered suitable. Bromate results from the reaction of O₃ and HO• with
242 bromide. NDMA can be formed from the reaction of amine precursors (e.g. containing
243 hydrazine, sulfamide, and dimethylamino functional groups) with generally low yields but that

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244 can reach up to $\geq 50\%$ in exceptional cases (Kosaka et al. 2009; Schmidt and Brauch 2008; von
245 Gunten et al. 2010; Krasner et al. 2013, Sgroi et al., 2014). Because precursors are mostly
246 unknown or unidentified in wastewater, the formation of NDMA cannot be excluded a priori.
247 NDMA can also already be present in the UWTP influent.

248 To minimize the release of biodegradable compounds including e.g. transformation products of
249 CECs formed during ozonation, a subsequent treatment by biologically active sand filtration
250 (or adsorption) is recommended. For the evaluation of the water quality after ozonation, specific
251 and unspecific toxicity of the treated wastewater needs to be measured with bioassays
252 (Schindler Wildhaber et al., 2015).

253

254 2.1.4 Application at full-scale as advanced treatment of urban wastewater

255 Ozonation is well established in drinking water treatment, but only recently has been applied at
256 full-scale as advanced treatment of urban wastewater in Europe for the removal of CECs before
257 discharge into the environment. In particular in Switzerland, ozonation is considered as one of
258 the BATs to meet the requirement of the new Swiss water protection Act (micropollutants
259 removal by 80% relative to the raw wastewater; Eggen et al. 2014, Bourgin et al. 2018), which
260 requires an upgrade of selected UWTPs until 2040. A website of the Swiss Water Association
261 provides updated information on European UWTPs that are planning or running full-scale
262 advanced treatment for CEC removal (www.micropoll.ch).

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263 The occurrence of organic matter (measured as DOC) and other readily oxidizable compounds
264 (such as nitrite) in the effluent of biological treatment affect ozone exposure and should be
265 considered when defining the ozone dose for the abatement of CECs. An ozone dose in the
266 range of 0.4 – 0.6 g O₃/g DOC (in the absence of nitrite) was found to be suitable to efficiently
267 abate micropollutants (Hollender et al. 2009, McArdell et al. 2015, Bourgin et al. 2018). Cost
268 evaluations are shown later (section 2.2.3) in comparison to treatment with activated carbon. In
269 the US and in Australia, ozonation followed by a BAC filter has been successfully applied as
270 low-cost potable reuse option (Gerrity et al. 2014; Reungoat et al. 2012; Stanford et al. 2017);

271

272 2.2 Activated Carbon adsorption

273 2.2.1 Removal of CECs

274 Unlike oxidation, adsorption is a separation process which does not result in the formation of
275 by-products. Activated carbon is the most used adsorbent in water treatment for the removal of
276 organic and inorganic pollutants dissolved in water. Activated carbon treatment for the removal
277 of CECs from wastewater has been widely investigated (Boehler et al., 2012; Grassi et al., 2013;
278 Rizzo et al., 2015; Ahmed, 2017; Kovalova et al., 2013, Michael et al., 2019). Packed bed
279 adsorption reactors with granular activated carbon (GAC) as adsorbent material are commonly
280 used in drinking water treatment. Due to process costs, their application at full-scale as
281 advanced urban wastewater treatment only recently has attracted the interest of UWTPs

282 managers and professionals, as the concern for possible effect on human health and
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283 environment of CECs has increased (Rizzo et al., 2019a; Siegrist et al. 2019). Its advantage
284 compared to powdered activated carbon (PAC) is that operationally it is easier to use, and it can
285 be recovered and regenerated when its adsorption capacity is exhausted. However, the process
286 requires an adequate monitoring strategy, since adsorption competition results in a reduced
287 CEC removal or even desorption of less adsorbable CECs with increasing treated bed volumes
288 due to a decrease in available adsorption sites. PAC can be applied as a post-treatment or dosed
289 into the biological unit in WWTs and, due to its smaller particle size (higher specific surface
290 area), is more efficient compared to GAC in the removal of water pollutants and specifically
291 CECs (Nowotny et al., 2007, Boehler et al., 2012).

292

293 2.2.2 Effect on ARB&ARGs

294 Even though adsorption is not a disinfection process and not designed to remove bacteria and
295 mobile genetic elements, a contribution to the reduction of antibiotic resistance in wastewater
296 effluent can be expected due to possible entrapment of ARB&ARGs inside the pores of
297 adsorbent particles (Zhang et al., 2017; Ashbolt et al., 2018; Bürgmann et al. 2018).

298

299 2.2.3 Application at full-scale as advanced treatment of urban wastewater

300 Activated carbon adsorption has been recently applied at full-scale for advanced treatment of
301 urban wastewater as alternative to ozonation, particularly in Switzerland and Germany, for the

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302 removal of CECs before effluent discharge into the environment (Rizzo et al., 2019a).
303 Depending on DOC and operation technology, a dose of 10-20 mg/L PAC can be recommended
304 to protect the aquatic environment (Boehler et al. 2012). A post-treatment is also needed in
305 PAC treatment for separation of residual PAC material. The use of GAC-packed reactors is
306 more restricted since it does not allow to react to certain conditions (e.g. rainy periods), whereas
307 PAC dose can be increased (Siegrist et al., 2019). However, GAC in combination with other
308 treatment is used successfully for many years, but just for direct potable reuse application
309 (Vaidya et al. 2019; Piras et al., 2020). As far as operation costs are concerned, feasibility
310 studies conducted in the state of North Rhine-Westphalia (Germany) in the years 2009–2016
311 resulted in similar median costs (0.04 €/m³) for ozonation (16 plants), PAC (11) and GAC (9)
312 processes (Figure SI4 in Rizzo et al., 2019a), with highest variability for GAC treatment.
313 Overall costs, including investment and operation, vary substantially with the size of the
314 UWTP. For mid-scale plants (~50.000 PE), the costs are in the range of 0.10 to 0.15 €/m³ treated
315 wastewater, decreasing further with increasing plant size even below 0.05 €/m³, with PAC
316 treatment being slightly more expensive than ozonation (Figure 4, Rizzo et al. 2019a).
317 Consistently with the numbers determined in Germany, overall costs for PAC (0.10-0.15
318 CHF/m³, 1 CHF being 0.88 € on January 18th, 2019, for dosing 10 mg/L PAC in a large plant
319 with 590,000 p.e.) were estimated to be higher than for ozonation (0.04-0.06 CHF/m³, for
320 dosing 5 mg/L ozone in a large plant) in Switzerland (McArdell et al., 2015, Abegglen et al.
321 2012).

322

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323 2.3 Chemical oxidants/disinfectants

324 Chlorination is by far the most common method of wastewater disinfection, but the concern for
325 human health and the environment related to the formation of toxic by-products (e.g.,
326 trihalomethanes, haloacetic acids and related contaminants) is increasing the interest towards
327 alternative chemical disinfectants, such as peracids. Among them, peracetic acid (PAA) already
328 finds different applications at full-scale in UWTPs, particularly in Italy (Formisano et al., 2016;
329 Di Cesare et al., 2016a) and in the USA (Bell and Wylie, 2016; Stewart et al., 2018).
330 Accordingly, chlorination and PAA disinfection are discussed in the subsequent sub-
331 paragraphs. Neither of the two technologies is applied for CEC abatement as they are not
332 economic and produce problematic effluents.

333

334 2.3.1 Chlorination

335 Wastewater disinfection by chlorine is typically performed by chlorine gas (in medium – large
336 UWTPs) or hypochlorite (either calcium or sodium). Limited studies have focused on the
337 abatement of CECs by chlorine, which was found to be quite poor, in particular if compared to
338 oxidation/disinfection processes with higher oxidation potential such as ozone and other AOPs
339 (Anumol et al., 2016; Hua et al., 2019). For example, Li and Zhang (2011) reported abatement
340 of antibiotics during wastewater treatment with chlorine in the range of 18% (roxithromycin)
341 to 40% (trimethoprim), while cephalixin and ampicillin were abated by 99% and 91%,
342 respectively. However, the chlorine dose was not reported in this study, and cephalixin and

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343 ampicillin are beta-lactam antibiotics that hydrolyze very quickly, so these results do not allow
344 to discriminate hydrolysis contribution from chlorine oxidation effects. Contrasting results are
345 documented in the scientific literature for sulfamethoxazole (SMX). Whilst Gao et al. (2014)
346 observed an almost complete abatement of SMX (initial concentration in the range 0.05–2
347 mg/L) within 15 min contact time and 2.0 mg/L of chlorine, de Jesus Gaffney et al. (2016)
348 observed only 20% abatement (pH 6–7, 2 mg/L of free chlorine) of SMX after 2 h contact time.
349 However, when reaction kinetics of SMX were investigated in different water matrices, the
350 results achieved in real wastewater ($[SMX]_0 = 2.0 \times 10^{-6}$ M), pH 7.3, free residual chlorine
351 (FRC) 11 mg/L) confirmed the substantial degradation of SMX observed in deionized water
352 (half-life of 23 s was measured under pseudo-first-order conditions ($[FRC]_0 = 20$ μ M (1.4
353 mg/L)) (Dodd and Huang, 2004). This expectation is supported by existing observations at full-
354 scale UWTs, where 89.6% SMX abatement was observed (Renew and Huang, 2004). Despite
355 the fact that single compounds are degraded by chlorination, a broad abatement of CECs cannot
356 be achieved; for example, poor or no abatement of diclofenac or carbamazepine was observed
357 (Hua et al. 2019).

358 Chlorination can result in the formation of toxic by-products, including trihalomethanes and
359 haloacetic acids (Richardson et al., 2007). Moreover, in effluents with incomplete nitrification,
360 chlorine combines with ammonia to form chloramines or so-called combined chlorine.
361 Chloramine chemistry is complex and will not be discussed further here, but it is noteworthy
362 that chloramines are weaker oxidants and disinfectants compared to free chlorine. NDMA is a
363 typical disinfection byproduct when chloramines are generated in wastewater effluents (Sgroui

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364 et al., 2018). It can be concluded that chlorination is not an option for CECs abatement and
365 could produce an adverse effect on effluent organic composition when used for disinfection.

366 The effect of chlorination on ARB is being investigated since the 70's (Grabow et al., 1976).
367 Although the chlorination process was found to effectively decrease antibiotic resistant *E. coli*
368 in wastewater, it may select bacterial population by increasing antibiotic resistant *E. coli* strains
369 compared to the corresponding total population (Fiorentino et al., 2015). However, when the
370 effect of chlorination on ARGs was investigated, different results were observed. For example,
371 ARGs *ereA* and *ermB* persisted in chlorinated (15 mg Cl₂ min/L) urban wastewater samples
372 (Yuan et al. 2015) and chlorination was found to be effective in ARGs removal (3.16 Log for
373 *suII* and 3.24 Log for *tetG* after 120 min treatment) only at non-realistic chlorine concentration
374 (160 mg/L) (Zhuang et al., 2015). On the opposite, Zheng and colleagues (2017) observed that
375 chlorination can reduce ARGs (*tetA*, *tetM*, *tetO*, *tetQ*, *tetW*, *suII* and *suIII*) abundance to some
376 extent (less than 1 Log unit for *tetA*) even under realistic operating conditions (5 mg/L of
377 chlorine, 30 min contact time). Moreover, Yoon et al. (2017) observed 4 Log reduction of ARGs
378 concentration (two differing amplicons located in the commercially available plasmid pUC4K
379 i.e., *amp*^R and *kan*^R) with 33-72 (mg·min)/L chlorine dose at pH 7 in urban wastewater. In
380 particular, intracellular ARGs showed lower rates of damage compared to the extracellular
381 ARGs, possibly due to the protective roles of cellular components. However, when process
382 efficiency was investigated in full-scale UWTPs, chlorination did not prove to have significant
383 contribution to ARGs (*tetA*, *tetW*, *tetO*, *ermB*, *qnrS*, *bla*_{TEM} *suII*) removal (Munir et al., 2011;
384 Gao et al., 2012; Di Cesare et al., 2016b).

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385

386 2.3.2 Disinfection with peracetic acid

387 PAA is a strong and broad-spectrum disinfectant, with a high reduction-oxidation (redox)
388 potential and strong biocidal effects on bacteria. Because of the formation of toxic by-products
389 in chlorination, PAA is increasingly replacing chlorine in UWTPs as it shows a broad-spectrum
390 efficiency and comparable way of application (Antonelli et al., 2013; Formisano et al., 2016;
391 Di Cesare et al., 2016a).

392 In spite of no significant formation of disinfection by products (DBPs) resulting from
393 wastewater disinfection by PAA when low doses are used (<5-10 mg/L) (Nurizzo et al., 2005),
394 PAA was found to be toxic for bacteria and crustaceans, even at concentrations lower than the
395 ones commonly used in wastewater disinfection (2-5 mg/L). But when PAA was compared to
396 other disinfection processes, a lower toxicity against aquatic organisms was observed. In
397 particular da Costa et al. (2014) compared PAA (5 mg/L, 20 min contact time), UV light
398 (average UV dose at 254 nm 670.8 mJ/cm², 120 s contact time), ozone (29.9 mg/L, 5 min
399 contact time), and sodium hypochlorite (2.5 mg/L, 20 min contact time) against *Ceriodaphnia*
400 *silvestrii*, *Daphnia similis*, *Chironomus xanthus*, and *Danio rerio* and toxicities after treatment
401 were in the order of free chlorine > ozone > UV > PAA after the respective disinfection
402 treatments had been applied to secondary effluent.

403 Due to its lower oxidation potential compared to ozone and hydroxyl radicals, possible
404 abatement of CECs in wastewater by PAA has not attracted the interest of the scientific

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405 community. As matter of fact, PAA effect on CECs has been investigated only as control test
406 compared to UV/PAA process (Rizzo et al., 2019b). Unlike carbamazepine (no abatement
407 observed even after 300 min contact time), diclofenac was effectively oxidized by 2 mg PAA/L
408 already after 60 min (80% abatement), while SMX was abated at a lower percentage (52% after
409 300 min). As PAA effect on ARB is of concern, the limit of detection was achieved within 15
410 min treatment in groundwater inoculated with an antibiotic resistant *E. coli* strain by 1 mg/L
411 and 2 mg/L of PAA (Rizzo et al., 2019b). However, the water matrix strongly affects bacterial
412 inactivation efficiency. As a matter of fact, Huang et al. (2013) observed lower inactivation in
413 reclaimed water with a higher PAA initial dose (20 mg/L). In particular, inactivation was higher
414 for ampicillin-resistant bacteria (2.3 Log) than for total heterotrophic bacteria (2.0 Log) and
415 tetracycline resistant bacteria (1.1 Log) after 10 min treatment. Moreover, the regrowth of
416 chloramphenicol-and tetracycline-resistant bacteria, as well as total heterotrophic bacteria was
417 more than 10-fold compared to those in the untreated wastewater sample (22 h stilling culture
418 after exposure to 2 or 5 mg PAA /L as for 10 min). Di Cesare et al. (2016a) evaluated the fate
419 of diverse ARGs, heavy metal resistant genes and of a mobile element (the class I integron) in
420 three UWTs using different disinfection processes. In 2 (*suIII* and *tetA*) out of 4 (*ermB* and
421 *qnrS*) of the quantified ARGs, a decrease was observed after PAA treatment.

422

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423 2.4 UV radiation

424 UV radiation (250-270 nm) is widely used for urban wastewater disinfection either for effluent
425 discharge or reuse (Munir et al., 2011; Di Cesare et al., 2016a). UV radiation can damage DNA,
426 resulting in the inhibition of cell replication and, in case of lethal doses, in loss of the ability of
427 reproduction. The effectiveness of a UV disinfection system depends on the characteristics of
428 the wastewater, the UV fluence (intensity \times irradiation time), the type of microorganisms and
429 reactor configuration. Since turbidity and suspended solids drastically decrease UV disinfection
430 efficiency, conventional depth filtration should be used before UV disinfection (not necessary
431 when applied following a membrane biological reactor (MBR)).

432

433 2.4.1 Abatement of CECs

434 UV radiation is not at all or is poorly effective in the abatement of most of CECs from water
435 and wastewater, but it can abate some antibiotics and other CECs at very high UV doses (Kim
436 et al., 2009; Rizzo et al., 2019b). For example, an almost complete abatement of tetracyclines
437 and ciprofloxacin was achieved but only at high UV doses (11,000-30,000 mJ/cm²) (Yuan et
438 al., 2011) and high abatement efficiencies (86-100%) were also observed for sulfonamides
439 (SMX and sulfadimethoxine) and quinolones (norfloxacin and nalidixic acid) (Kim et al.,
440 2009). Iodinated X-ray contrast media were abated by more than 90% at 720 mJ/cm² (Kovalova
441 et al. 2013).

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442

443 2.4.2 Effect on ARB&ARGs

444 The effect of UV radiation on ARB&ARGs in urban wastewater has been increasingly
445 investigated in the last years at lab and full-scale (Munir et al., 2011; McKinney and Pruden,
446 2012; Rizzo et al., 2013; Guo et al., 2013; Zhuang et al., 2015; Di Cesare et al., 2016a). Process
447 efficiency strongly depends on the applied UV dose and target ARB&ARGs, and possibly this
448 is the main reason to explain differences between lab- and full-scale evidences.

449 Efficient removal of heterotrophic bacteria harboring resistance to erythromycin and
450 tetracycline was observed (Guo et al., 2013) (equivalent Log reduction being 1.4 and 1.1 at a
451 UV dose of 5 mJ/cm²). As UV dose was further increased to 20 and 50 mJ/cm², respectively,
452 ARB were below the detection limit (1 CFU/mL).

453 The UV dose also affects the removal of ARGs. UV doses ranging from 200 to 400 mJ/cm² (at
454 least one order of magnitude higher than those for the inactivation of host bacterial cells) were
455 required to remove 3 or 4 Log units of ARGs, namely *ampC*, *mecA*, *tetA* and *vanA* (McKinney
456 and Pruden, 2012). Actually, also lower UV doses (5-10 mJ/cm²) were found to be effective in
457 the removal of ARGs (namely *ereA*, *ereB*, *ermA*, *ermB*, *tetA*, *tetO*) but starting from lower
458 initial ARGs copies per mL (Guo et al., 2013). The relative abundance of selected ARGs
459 increased with low doses of UV (Zhuang et al., 2015). Less than one order of magnitude
460 removal of five tetracycline resistance genes (*tetA*, *tetM*, *tetO*, *tetQ*, *tetW*) and two sulfonamide
461 resistance genes (*suII*, *suIII*) were observed in UV disinfection (UV fluence 10-160 mJ/cm²) of

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462 wastewater samples taken from the secondary sedimentation tank of a UWTP in Hangzhou,
463 China (Zheng et al., 2017). The removal efficiency of the five *tet* genes was between 52.0%
464 and 73.5% at the lower fluence UV disinfection (40 mJ/cm² or less), and between 79.7%, and
465 92.0% at high fluence (160 mJ/cm²). Lower removal efficiencies were observed for *sulI*, *sulII*
466 (78.1% and 71.1% respectively, at the higher fluence).

467 In full-scale monitoring (5 UWTPs in the USA), UV radiation employed for disinfection did
468 not prove to have a significant contribution to ARGs (*tetw*, *tetO*, *sulI*) and ARB reduction
469 (Munir et al., 2011). These results were confirmed in a subsequent study at full-scale, where no
470 significant difference in ARGs (namely, *ermB*, *qnrS* and *tetA*) was observed before and after
471 UV disinfection, while for *sulIII* even an increase was observed after disinfection (Di Cesare et
472 al., 2016a).

473

474 2.5 Advanced oxidation processes

475 Advanced oxidation processes (AOPs) rely on the formation of hydroxyl radicals that can abate
476 a wide range of CECs (Rizzo, 2011; He et al., 2020) as well as inactivate microorganisms
477 (Dunlop et al., 2010; Fiorentino et al., 2015). A possible classification of AOPs includes two
478 groups: homogeneous processes (e.g., UV/H₂O₂, UV/Fe/H₂O₂, O₃, O₃/H₂O₂ etc.) and
479 heterogeneous (solid semiconductors + light source, e.g., UV/TiO₂, UV/ZnO) photocatalytic
480 processes. Homogeneous processes have been widely investigated as advanced treatment of
481 urban wastewater effluents and either are already applied at full-scale (e.g., O₃, see section 2.1)

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482 or are characterized by short-/mid-term perspective application (e.g., UV/H₂O₂, UV/Fe/H₂O₂)
483 as opposed to heterogeneous photocatalytic processes (Rizzo et al., 2019a; Maniakova et al.,
484 2020). The main reason why heterogeneous photocatalytic processes are not ready for full-scale
485 application as advanced urban wastewater treatment are related to photocatalyst preparation
486 costs, photocatalyst quantum yield (effectiveness) and reactor configuration (Iervolino et al.,
487 2020). In particular, heterogeneous photocatalytic processes can be operated under two main
488 configurations: (i) with the photocatalyst suspended in the reactor (i.e., slurry system) or (ii)
489 attached to a support (i.e., immobilized system). Due to the higher specific surface area
490 available, a slurry system is more effective than an immobilized one, but a subsequent
491 expensive separation process (e.g., coagulation, filtration, membrane) is necessary to recover
492 the photocatalyst before effluent discharge or reuse (Fernández-Ibáñez et al., 2003).
493 Immobilized photocatalytic systems have relatively lower quantum efficiency than slurry ones,
494 which results in longer treatment time and consequently larger water volume to treat (Spasiano
495 et al., 2015). Some homogeneous photo-driven AOPs can also be operated under natural
496 sunlight (solar/H₂O₂ or solar/Fe/H₂O₂) thus saving energy costs (Klamerth et al., 2010; Ortega-
497 Gomez et al., 2014; Ferro et al., 2015; Giannakis et al., 2016) and this can be considered as an
498 attractive option for small UWTs in areas with sufficient sunlight.

499

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500 2.5.1 Abatement of CECs

501 Due to their high redox potential hydroxyl radicals oxidize a wide spectrum of organic
502 contaminants, accordingly, AOPs successfully degrade several organic micropollutants
503 (Klavarioti et al, 2009; Rizzo, 2011). The most common AOPs studied are UV/H₂O₂, O₃/H₂O₂,
504 O₃/UV, Fenton (Fe/H₂O₂), photo-Fenton (UV/Fe/H₂O₂) and heterogeneous photocatalysis (e.g.,
505 UV/TiO₂, UV/ZnO). Although UV/H₂O₂, is more efficient than UV alone to abate CECs, still
506 more energy is needed compared to ozonation (Rizzo et al., 2019a). O₃/H₂O₂ does not improve
507 abatement of CECs compared to ozone alone in UWTP effluents, since effluent ozonation can
508 be considered an intrinsically AOP due to the high HO[•] generation potential of the organic
509 matrix (Buffle et al., 2006), at the same time HO[•] are scavenged by the matrix (Acero and von
510 Gunten, 2001; Kovalova 2013). Fenton and photo-Fenton processes are typically effective
511 under acidic conditions (pH 3) and the abatement of three antibiotics, namely SMX,
512 erythromycin (ERY) and clarithromycin, from urban wastewater was investigated (Karaolia et
513 al., 2017). SMX and ERY were efficiently abated from UWTP secondary effluents by solar
514 photo-Fenton in continuous flow operation with >80% abatement at a hydraulic residence time
515 of 20 min in non-concentrating raceway pond reactors (Arzate et al., 2017). Nonetheless, this
516 operation mode at full-scale would result in additional process cost and salinity increase
517 because pH has to be first decreased and subsequently neutralized before effluent discharge or
518 reuse. However, photo-Fenton has also been successfully investigated under almost neutral pH
519 conditions and solar radiation for the abatement of CECs from urban wastewater with the
520 addition of complexing agents. As a matter of fact, the (solar driven) photo-Fenton process

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521 allowed to effectively decrease CECs from urban wastewater under so-called mild conditions,
522 i.e. under low Fe (< 5 mg/L) and H₂O₂ (< 20 mg/L) concentrations and pH 5-6, thus avoiding
523 the necessity for final separation of soluble iron species from the treated wastewater (Klammerth
524 et al., 2010; De la Obra et al., 2017). The use of organic chelating agents makes the process
525 feasible and effective even under neutral pH conditions (De Luca et al., 2014; Fiorentino et al.
526 2018; Soriano-Molina et al., 2018). Unlike photo-Fenton, solar-UV/H₂O₂ process can be
527 operated at neutral pH without chelating agents, and it can successfully abate some CECs, but
528 longer reaction time compared to photo-Fenton is needed (Ferro et al., 2015).

529

530 2.5.2 Effect on ARB&ARGs

531 AOPs can successfully inactivate ARB in urban wastewater (Karaolia et al., 2014; Rizzo et al.,
532 2014a; Fiorentino et al., 2019). As a matter of fact, sunlight/H₂O₂ process resulted in a total
533 inactivation of multi drug resistant (MDR) *E. coli* (resistant to a mixture of three antibiotics:
534 ampicillin, ciprofloxacin and tetracycline), after 90 min of treatment (Fiorentino et al., 2015).
535 Noteworthy, longer treatment time (120 min) was necessary to achieve a complete inactivation
536 of the total *E. coli* population, despite the percentage of MDR *E. coli* ((total *E. coli* – MDR *E.*
537 *coli*)x100/total *E. coli*) increased as total *E. coli* population decreased with treatment time.

538 However, the release of mobile genetic elements from bacterial cells, that may take place after
539 disinfection process, and the potential to transfer antibiotic resistance through horizontal
540 mechanism, have been poorly investigated. Photo-driven AOPs have recently been investigated

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541 to evaluate if they can be more effective in the removal of ARGs than conventional disinfection
542 processes, such as chlorination and UV radiation. Ferro et al. (2016) investigated the effect of
543 UV/H₂O₂ (broad-band spectrum UV lamp with main emission in the range 320-450 nm), under
544 realistic conditions for wastewater treatment (natural pH (7.6) and 20 mg H₂O₂/L), on antibiotic
545 resistance transfer potential in urban wastewater. The investigated process resulted in bacterial
546 inactivation and a decrease of ARGs in intracellular DNA after 60 min treatment, but UV/H₂O₂
547 did not remove ARGs effectively. Actually, an increase up to 3.7×10^3 copies/mL ($p > 0.05$)
548 of *bla*_{TEM} gene was observed in total DNA after 240 min treatment, while no difference ($p >$
549 0.05) was found for *qnrS* gene between the initial (5.1×10^4 copies/mL) and the final sample
550 (4.3×10^4 copies/mL). In UV/H₂O₂ process (pH 7, 50-130 mJ/cm²), 4 Log reduction of ARGs
551 (*amp*^R and *kan*^R) concentration was observed in urban wastewater (Yoon et al., 2017).
552 According to the results previously discussed for the chlorination process, intracellular ARGs
553 showed lower rates of damage compared to extracellular ARGs due to cell protective roles and
554 significant HO[•] radical scavenging by cellular components. Zhang et al. (2016a) showed that
555 UV/H₂O₂ can effectively remove ARGs (2.8-3.5 logs removal of *sul1*, *tetX*, and *tetG*, within
556 30 min treatment) but only under conditions that seem unrealistic for full-scale implementation
557 (pH 3.5 and 340 mg H₂O₂/L), moreover UV fluence was not provided.

558 Solar driven photo-Fenton process is effective in the inactivation of ARB Karaolia et al., 2017;
559 Fiorentino et al., 2019). When the process (5 mg Fe²⁺/L, 50 mg H₂O₂/L, pH 3) was operated at
560 pilot scale through a compound parabolic collector (CPC) based reactor, on the effluent of an
561 MBR, a complete inactivation of the low initial bacterial population (*E. coli* = 2 CFU/100 mL,

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562 *P. aeruginosa* = 4 CFU/100 mL, *Klebsiella* spp. = 3 CFU/100 mL), including antibiotic-tolerant
563 and susceptible bacteria, was observed, after 54 min of solar radiation intensity normalized time
564 (Karaolia et al., 2017). On the other hand, repair of *P. aeruginosa* was observed, with 2
565 CFU/100 mL growing on the selective media 24 h after solar Fenton oxidation. Solar photo-
566 Fenton process was also investigated in raceway pond reactors, at neutral pH conditions (20 mg
567 Fe²⁺/L, 50 mg H₂O₂/L), in real urban wastewater and an effective inactivation of *E. coli* and
568 *Enterococcus* sp. cefotaxime resistant bacteria was observed (detection limit (1 CFU/mL)
569 achieved after 30-40 min, 3.2-4.7 kJ/L) (Fiorentino et al., 2019). However, both solar driven
570 photo Fenton processes (CPC reactor at pH 3 and raceway ponds at neutral pH) did not
571 effectively remove the target ARGs..

572 The effect of heterogeneous photocatalysis with TiO₂ on ARB&ARGs has been investigated in
573 slurry and immobilized systems. According to the results observed for homogenous photo-
574 driven AOPs, even heterogeneous photocatalytic processes, while effective in the inactivation
575 of different antibiotic resistant bacterial populations (Tsai et al., 2010; Rizzo et al., 2014a, Rizzo
576 et al., 2014b; Dunlop et al., 2015; Zammit et al., 2019) may not be effective in the removal of
577 some ARGs (Karaolia et al., 2018).

578

579 2.6 Membrane filtration

580 Membrane separation processes include microfiltration (MF), ultrafiltration (UF),
581 nanofiltration (NF) and reverse osmosis (RO), which may be operated separately or in

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582 combination with other processes as a part of integrated technologies such as MBR. NF and
583 RO are effective in the removal of both organic and inorganic CECs (Bellona et al., 2004;
584 Alturki et al., 2010; Garcia et al., 2013), while MF or UF are typically used as pre-treatment of
585 either NF or RO to control membrane fouling as well as for disinfection and solids removal.
586 NF and specifically RO provide the opportunity to reduce the effluent salinity, which can be
587 necessary depending on the downstream application of the treated effluent. However, a waste
588 stream containing the separated salts and other pollutants is generated as well.

589

590 2.6.1 Removal of CECs

591 Removal of CECs by membrane processes is primarily based on size exclusion, although
592 electrostatic interactions between charged solutes and negatively charged membranes typically
593 have an important role in the removal (Bellona et al., 2004). Hydrophobic trace contaminants
594 have been shown to adsorb to membrane surfaces reducing the rejection of these contaminants
595 through both RO and NF. This has been shown to be particularly relevant in NF processes.
596 Several other factors typically also affect the removal of the target CECs (such as phenolic
597 aromatic compounds) by membrane processes (Bellona et al., 2004). Depending on the type of
598 membrane, the range of rejections of CECs by both RO and NF is quite broad, but the rejection
599 can be higher than 99% for high rejection RO membranes (Krzeminski et al., 2017). However,
600 in these membrane processes the CECs are accumulating in the rejected concentrate. The
601 discharge of the concentrate to the environment can be problematic, as the original salt and

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602 pollutant load of the secondary effluent, while not having increased in absolute mass, is now
603 concentrated typically by a factor of 3 to 7, depending on the permeate water recovery
604 percentage of the membrane process. The presence of the contamination in concentrated form
605 can also be an opportunity for targeted treatment since pollutants are more effectively treated
606 by advanced oxidation processes (usually governed by first order kinetics) as initial
607 concentration increases (Miralles-Cuevas et al., 2016).

608 Full-scale applications of RO technology are reported in potable reuse treatment trains, e.g. the
609 Orange County Groundwater Replenishment System (California, USA), NEWater facilities at
610 the Bedok, Kranji, Ulu Pandan and Changi facilities in Singapore and the Torreele Reuse
611 Facility in Belgium (Raffin et al., 2013; Gerrity et al. 2013). RO is also used in direct potable
612 reuse treatment trains, along with MF or UF, in Cloudcroft (New Mexico) and Big Spring
613 (Texas) in USA (Gerrity et al. 2013). NF typically removes CECs in the 300-1,000 molecular
614 weight (MW) range, rejecting selected salts and most organic constituents and microorganisms,
615 operating at higher recovery rates and lower pressures than RO processes. Accordingly, and
616 when feasible, NF can be used instead of RO to save some energy, chemical and concentrate
617 disposal costs (Yangali-Quintanilla et al., 2010). While offering very high removal efficiencies
618 for CECs, specifically RO, on the downside these technologies exhibit high energy
619 consumption.

620

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621 2.6.2 Effect on ARB&ARGs

622 As the separation principle is purely based on size, the removal of ARB can be expected to
623 behave very similar to the removal of those not carrying antibiotic resistance. MF and UF are
624 commonly applied barriers for pathogens, with MF being very effective against protozoa and
625 bacteria, while due to a larger pore size, it is not very effective in removing viruses. UF removes
626 all three classes of pathogens to a very high extent (2 to 4 Log removal values (LRV)) (Hai et
627 al. 2014). NF and RO membranes present in theory an even smaller pore size and should be
628 “perfect filters”. In fact, > 6 LRV virus removal has been observed at pilot-scale. However, due
629 to the modular engineering approach system breaches cannot be per se excluded and finding
630 appropriate surrogate measurements remains a challenge to ensure disinfection during
631 operation, at least at levels beyond e.g. the removal of electrical conductivity (Pype et al, 2016).

632 The effect of membrane filtration, in particular NF and RO, on ARB&ARGs, thus far, has been
633 little discussed in the literature as the existing studies have focused mostly on MBRs and MF
634 and UF membranes (Munir et al. 2011; Riquelme Breazeal et al., 2013; Rizzo et al., 2013; Yang
635 et al., 2013; Sun et al., 2016; Threedeach et al., 2016; Li et al., 2019).

636 As previously mentioned, membranes can remove bacteria due to membrane retention, thus
637 contributing to reducing the spread of multiple antibiotic resistant strains (Verlicchi et al. 2015).
638 For example, filtration of ARGs spiked UWTP effluent through the 100, 10 and 1kDa
639 membranes in the lab-scale stirred ultrafiltration cell reduced *vanA* and *bla_{TEM}* ARGs by 0.9,
640 3.5 and 4.2 Log, respectively (Riquelme Breazeal et al., 2013). The removal of plasmid-

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641 associated ARGs improved further at the presence of colloidal material in the water matrix and
642 the colloids influence became more apparent as the membrane pore size decreased. The DNA
643 removal was attributed to membrane retention and following mechanisms: i) size exclusion of
644 the DNA, ii) size exclusion of DNA-colloid complexes, or iii) interactions with the membrane
645 material (Riquelme Breazeal et al., 2013).

646 Arkhangelsky et al. (2008, 2011) studied, in lab-scale dead-end membrane cell, penetration of
647 plasmid DNA through UF membranes and demonstrated that despite electrostatic repulsion and
648 a significant size difference between plasmid and pore sizes, DNA can penetrate through the
649 UF membrane, indicating that UF did not provide absolute barrier for DNA retention. Also,
650 Riquelme Breazeal et al. (2013) observed that 1 kDa membrane did not completely retain
651 plasmid and pointed out that the effective size of DNA is smaller than predicted by molecular
652 weight because DNA is a long, thin and flexible molecule. Although the penetration mechanism
653 is not yet clear, Arkhangelsky et al. (2011) suggested that plasmid stretches into long hair-
654 shaped flexible strands and penetrates pores based on ‘snake-like’ movement due to
655 hydrodynamic pressure (transmembrane pressure, TMP) with gradual pore blocking. The
656 proposed penetration mechanism is in accordance with the findings of other studies on DNA
657 (Marko et al., 2011; Travers, 2004). In addition, plasmid transportation levels are linearly
658 correlated to the TMP.

659 Böckelmann et al. (2009) studied three artificial recharge systems in Europe. Combination of
660 UF and RO proved to be an efficient barrier for the elimination of ARGs. ARGs *tetO* and *ermB*

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661 detected in UWTP effluent at concentrations of $1.05 \times 10^7 \pm 3.54 \times 10^6$ gene copies/100mL and
662 $1.92 \times 10^5 \pm 1.06 \times 10^4$ gene copies/100mL, respectively, were removed during the UF-RO
663 process applied in the Torreele Reuse Facility. Noteworthy, *tetO* were detected again, at low
664 concentrations, in subsequent sampling points: in the infiltration water before transport
665 ($5.92 \times 10^3 \pm 1.39 \times 10^3$ gene copies/100mL) and in the groundwater after infiltration (3.13×10^3
666 $\pm 1.52 \times 10^3$ gene copies/100mL). In a recent work, a wastewater reuse treatment train including
667 MBR with MF membranes followed by RO provided up to 3.8 Log removal of the ARGs down
668 to absolute abundance of 4.03×10^4 copies/mL (Lu et al., 2020). MF was capable of 2-3 Log
669 removal of ARGs whereas subsequent RO provided additionally up to 1.5 Log removal.
670 Another recent full-scale study investigating the removal of ARGs in a full-scale wastewater
671 treatment plant including biological and physicochemical treatment located on a swine farm
672 showed very high removals for ARGs in both, NF and RO. The removals achieved depended
673 on the ARG and ranged from 5 to 8 Log removals compared to raw sewage (Lan et al., 2019).
674 Above 99.2% removal of free DNA from UWTP effluent by NF membrane in the lab-scale
675 system was reported (Slipko et al., 2019). Similar removal rates were observed both in water
676 and in effluent. According to the authors, besides size exclusion mechanism, electrostatic
677 repulsion plays also important role in removal of free DNA in NF and RO.

678

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679 2.7 Comparison among BATs for the removal of CECs relevant for crop uptake

680 During the last years, several classes of CECs have been proven to taken up through roots and
681 translocated to the aerial parts of crop plants irrigated with treated wastewater, grown under
682 hydroponic or greenhouse control conditions, as well as soils irrigated with treated wastewater
683 in real agricultural systems. The uptake is largely dependent on CECs' bioavailability in soil
684 pore water near the rhizosphere and thus on their physicochemical properties and the properties
685 of the soil environment. Once taken up, the transport of CECs within the plant vascular
686 translocation system (xylem and phloem) mainly depends on their lipophilicity and electrical
687 charge, as well as the physiology and transpiration rate of crop plants and environmental
688 conditions (i.e. drought stress), (Nereus COST Action ES1403, Deliverable 11). Accordingly,
689 different crops have different potential for CECs uptake, for example, uptake potential is
690 generally higher for leafy vegetables compared to fruit vegetables or cereal crops. The main
691 biotic factors that may affect the uptake of CECs by plants are the plant itself (including the
692 species, the variety and cultivar, the genotype, and the physiological state of the plant), and the
693 soil fauna, which constitute the main cause for the biodegradation and biotransformation of
694 CECs within the soil (Ahuja et al., 2010; Goldstein et al., 2014). Climatic conditions and other
695 environmental perturbations (such as temperature, wind speed, UV radiation, salinity, drought,
696 environmental pollution, etc.) constitute the main abiotic factors that influence the potential for
697 CECs uptake by crop plants (Dodgen et al., 2015; Zhang et al., 2016b). The majority of studies
698 with regard to CECs uptake, either conducted in controlled laboratory or greenhouse conditions
699 or under field or simulated conditions, employed mostly (a) vegetables (leafy vegetables such

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700 as lettuce and cabbage, fruit vegetables such as tomato and cucumber, and root vegetables such
701 as carrot and radish) and (b) cereals and fodder crops (i.e. maize, wheat, alfalfa). Experimental
702 results revealed that the potential for CECs uptake by crop plants decreased in the order of leafy
703 vegetables > root vegetables > cereals and fodder crops > fruit vegetables. Though, the uptake
704 of CECs by important crop plants, such as fruit trees, has not yet been evaluated. Fruit trees,
705 such as citrus, bananas, apple and other fruit bearing trees, have high net irrigation requirements
706 and evapotranspiration rates, which may render them as plants with moderate to high potential
707 for CECs uptake (similar to that of fruit vegetables), (Christou et al. 2019). Therefore, the
708 recommendation on the BAT should consider both the soil and the type of the crop species to
709 be irrigated by reclaimed water.

710 Consistently with the aim of the present review paper, a comparison among the above-
711 mentioned BATs was performed according to the chemical CECs relevant for crop uptake by
712 considering results from investigations at pilot or full-scale on real wastewater. According to
713 the list compiled by NEREUS COST Action ES1403, 27 CECs are relevant for crop uptake
714 (Krzeminski et al., 2019). The Action also applied selected criteria to establish a prioritised list
715 with CECs which include the following: 1) high frequency of detection in treated effluents,
716 which is related to high patterns of use and recalcitrance during the wastewater treatment
717 process, 2) environmental, agricultural and/or health concern; at least one of the following
718 criteria should be met by the target CECs: a) DT₅₀ (time necessary to degrade the 50% of the
719 original contaminant concentration) in soil > 14 d, b) phytotoxicity at environmental relevant
720 concentrations, c) promote a selection pressure to soil microbiota, d) potential human health

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721 effects according to threshold contaminant concentration criteria, 3) significant uptake rate by
722 crops (usually bioconcentration factors ($RCF = [root]/[growing\ medium]$; $LCF =$
723 $[leaf]/[growing\ medium]$; $FCF = [fruit]/[growing\ medium]$) higher than 1). The list of
724 prioritised CECs includes carbamazepine (CBZ), diclofenac (DCF), enrofloxacin, SMX, 17^α-
725 ethinyl estradiol, lamotrigine and trimethoprim, (Nereus COST Action ES1403, Deliverable 7;
726 Boxall et al., 2012; Calderón-Preciado et al., 2012; Christou et al., 2017b; Goldstein et al., 2014; Miller
727 et al., 2016; Tanoue et al., 2012; Wu et al., 2015; Zhang et al., 2016b). However, out of 27 crop
728 relevant CECs only for 3 compounds, namely CBZ, DCF and SMX, literature was found on
729 their removal from wastewater matrices during different advanced technologies (Table 1). For
730 SMX, high removal efficiencies (>80-100%) were observed during RO and NF, UV radiation,
731 chlorination (HOCl), ozonation and other AOPs, while lower efficiencies (<64%) were
732 observed for PAA and PAC treatment. High DCF removal efficiencies (80-100%) were
733 observed during RO and NF, UV radiation, PAA treatment, ozonation and other AOPs, good
734 removals ($\cong 70\%$) for PAC, lower (60%) for chlorination. Finally, high CBZ removal
735 efficiencies (90-100%) were observed for PAC, ozonation, and RO, a wide range of efficiencies
736 (>24-100%) for AOPs and NF, depending on the process and operating conditions, UV
737 radiation resulted in a poor efficiency (16%), and no removal was observed for chlorination and
738 PAA treatment under the investigated conditions.

739

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740 **Table 1.** Effect of BATs on the abatement of chemical CECs relevant for crop uptake. Only
 741 results from investigations at pilot or full-scale on real wastewater are presented (part of these
 742 data is extracted from Table 3 and supplementary information of “Rizzo et al., 2019a”).

CEC	Process	Scale of study	Water matrix ¹	DOC (mg/L)	CEC initial concentration	Comments	CEC abatement (%)	Reference
Sulfamethoxazole	PAC	Pilot/full	RMW	5-10	171 ng/L (data only from 1 paper)	10-20 mg PAC/L. 0.3-1h contact time.	58-64	Boehler et al. 2012; Margot et al. 2013
	GAC	Pilot	RMW	5.8	145 ng/L	7400 bed volumes treated. 14 min EBCT.	59	Bourgin et al. 2018
	O ₃	Pilot/full	RMW	3.5-8.6	-	0.61±0.04 g O ₃ /g DOC.	94-97	Hollender et al. 2009; Kreuzinger et al. 2015; Bourgin et al. 2018.
	Free chlorine	Full	RMW	-	576 ng/L	Neutral pH, sample taken from the effluent of chlorination unit (dose not provided)	89.6	Renew and Huang, 2004
	PAA	Pilot	RMW	24	100 µg/L	2.0 mg PAA/L, 300 min	52	Rizzo et al., 2019b
	UV	Pilot	RMW	24	100 µg/L	4.58 kJ/L	100	Rizzo et al., 2019b
	Solar photo-Fenton (CPC reactor)	Pilot	RMW/SR MW	10.2-42.7	5.5 ng/L – 1879 µg/L	Fe: 5 – 10 mg/L; H ₂ O ₂ : 20 – 100 mg/L; pH: 2.8 or higher (5-6).	>80-100	Klamerth et al., 2010; Karaolia et al., 2014, 2017; Prieto-Rodríguez et al., 2013;
	Solar photo-Fenton (Raceway pond)	Pilot	RMW	40	282 ± 36.7 ng/L	Continuous mode. Two liquid depths (5, 15 cm) and three HRTs (80, 40, 20 min); Fe: 5.5 mg/L; H ₂ O ₂ : 30 mg/L. pH 2.8.	81-100	Arzate et al., 2017

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Photo Fenton	Pilot	RMW	5-7.5 ²	487 ng/L	30 mg H ₂ O ₂ /L; 2 mg Fe/L. pH 6-7 (no chelating agents added). 5 low pressure mercury lamps (254 nm) of 150 W each, incident light 70 W/m ² .	82	De la Cruz et al., 2013
UV/H ₂ O ₂	Pilot	RMW	5-7.5 ²	487 ng/L	30 mg H ₂ O ₂ /L. 5 low pressure mercury lamps (254 nm) of 150 W each, incident light 70 W/m ² .	89	De la Cruz et al., 2013
sunlight/TiO ₂ (CPC reactor)	Pilot	SRMW	13	100 µg/L	TiO ₂ immobilized on glass spheres (0.335 g TiO ₂ /L). k=0.03 1/min	100	Miranda-García et al. 2011
RO	Pilot	Secondary treated wastewater r	-	56 ng/L	Saehan 4040 FL, Flux = 20 L/(m ² .h)	>98	Snyder et al. 2007
		RMW/primary treated wastewater r	7.8	15-1800 ng/L	Saehan 4040 FL, Osmonics AK4040, Flux = 17-20 L/(m ² .h)	94-99 (based on 2 studies)	
		Secondary/Tertiary treated wastewater r	-	805-1030 ng/L	Hydranautics ESPA2	>99 (based on 2 studies)	
RO	Pilot	RMW	-	85-122 ng/L	Filmtec TW30 25-40, Flux = 22-31 L/(m ² .h)	98	Sahar et al 2011
					Filmtec BW30-400, Flux = 45 L/(m ² .h)	98	
RO	Pilot	RMW	-	20-27 ng/L	Ropur TR70-4021-HF	>99	Dolar et al. 2012
NF	Pilot	RMW	-	100-500 ng/L	Filmtec NF90, MWCO 200 Da, Flux = 18 L/(m ² .h)	99	Mamo et al. 2018
						100	

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	RO					Hydranautics ESPA2, MWCO 100 Da, Flux = 18 L/(m ² .h)		
Diclofenac	PAC	Pilot	RMW	7.3(±1.9)	1187 ng/L	10-20 mg PAC/L; 0.3-0.7h contact time.	69	Margot et al. 2013
	GAC	Pilot	RMW	4.4	1008 ng/L	23400 bed volumes treated. 14 min EBCT.	72	Bourgin et al. 2018
	O ₃	Pilot/full	RMW	3.5-8.6	-	0.61(±0.04) g O ₃ /g DOC.	98-100	Hollender et al. 2009; Kreuzinger et al. 2015; Bourgin et al. 2018.
	Free chlorine	Full	RMW	-	-	Neutral pH	60	Anumol et al., 2016
	PAA	Pilot	RMW	24	100 µg/L	2.0 mg PAA/L, 60 min	80	Rizzo et al., 2019b
	UV	Pilot	RMW	24	100 µg/L	2.22 kJ/L	90	Rizzo et al., 2019b
	Photo-Fenton	Pilot	RMW	5-7.5 ²	925 ng/L	20-50 mg H ₂ O ₂ /L; 2-4 mg Fe/L. pH 6-7. 5 low pressure mercury lamps (254 nm) of 150 W each, incident light 70 W/m ² .	93-100	De la Cruz et al., 2013
	Solar photo-Fenton (CPC reactor)	Pilot	RMW/SR MW	10.2-36	1 – 5100 µg/L	Fe: 5 – 10 mg/L; H ₂ O ₂ : 20 – 60 mg/L; pH: 2.8 or neutral (chelating agent used).	80-100	Klamerth et al., 2010, 2011; Prieto-Rodríguez et al., 2013;
	UV/H ₂ O ₂	Pilot	RMW	5-7.5 ²	925 ng/L	20-50 mg H ₂ O ₂ /L. 5 low pressure mercury lamps (254 nm) of 150 W each, incident light 70 W/m ² .	99-100	De la Cruz et al., 2013

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	sunlight/TiO ₂ (CPC reactor)	Pilot	RMW/SR MW	13-23	414 ng/L-100 µg/L	20 mg/L TiO ₂ and supported TiO ₂ , neutral pH.	80-100	Miranda-García et al., 2011; Prieto-Rodríguez et al., 2012;
	RO	Pilot	Secondary treated wastewater r	-	37 ng/L	Saehan 4040 FL, Flux = 20 L/(m ² .h)	>97	Snyder et al. 2007
			RMW/primary treated wastewater r	7.8	1.1-38 ng/L	Saehan 4040 FL, Osmonics AK4040, Flux = 17-20 L/(m ² .h)	>93% (from 2 pilots)	
			Secondary/Tertiary treated wastewater r	-	49-59 ng/L	Hydranautics ESPA2	>98 (from 2 pilots)	
	RO	Pilot	RMW	-	500-580 ng/L	Filmtec TW30 25–40, Flux = 22-31 L/(m ² .h) Filmtec BW30–400, Flux = 45 L/(m ² .h)	95-99 (from 2 pilots)	Sahar et al 2011
	NF	Pilot	Effluent UWTP	-	720 ng/L	Flux = 1-2 LMH, TMP = 0.7 bar	60-65	Röhricht et al. 2009, 2010
	NF	Pilot	RMW	-	260-440 ng/L	FILMTEC NF90-4040, 200 Da	87-98	Cartagena et al. 2013
	RO					FILMTEC BW30-4040	88-96	
	NF	Pilot	RMW	-	100-500 ng/L	Filmtec NF90 MWCO=200 Da, Flux = 18 L/(m ² .h)	100	Mamo et al. 2018
	RO					Hydranautics ESPA2 MWCO 100 Da, Flux = 18 L/(m ² .h)	100	
Carbamazepine	PAC	Pilot/full	RMW	5-10	221-461 ng/L	10-20 mg PAC/L; 0.3-1h	90-92	Boehler et al., 2012; Margot

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						contact time; data from 3 papers.	et al., 2013; Mailler et al., 2015; Karelid et al., 2017.
GAC	Pilot	RMW	4.4	110 ng/L	23400 bed volumes treated. 14 min EBCT.	72	Bourgin et al. 2018
O ₃	Pilot/full	RMW	3.5-7.6	-	0.61±0.04 g O ₃ /g DOC.	97-100	Hollender et al. 2009; Kreuzinger et al. 2015; Bourgin et al. 2018.
Free chlorine	Full	RMW	-	-	Neutral pH	No removal	Anumol et al., 2016
PAA	Pilot	RMW	24	100 µg/L	2.0 mg PAA/L, up to 300 min	No removal	Rizzo et al., 2019b
UV	Pilot	RMW	24	100 µg/L	15.12 kJ/L	16	Rizzo et al., 2019b
Solar photo- Fenton (CPC rector)	Pilot	RMW/SR MW	10-36	70 ng/L- 100 µg/L	Fe: 5 mg/L; H ₂ O ₂ : 50 – 60 mg/L; pH: 2.8 or neutral (chelating agent used).	>24-100	Klamerth et al., 2010, 2011; Prieto- Rodríguez et al., 2013;
Solar photo- Fenton (Raceway pond)	Pilot	RMW	40	422 ± 54.9 ng/L	Two liquid depths (5, 15 cm) and three HRTs (80, 40, 20 min); Fe: 5.5 mg/L; H ₂ O ₂ : 30 mg/L. pH 2.8	86-96	Arzate et al., 2017
Photo- Fenton	Pilot	RMW	5-7.5 ²	333 ng/L	20-50 mg H ₂ O ₂ /L; 2-4 mg Fe/L. pH 6-7. 5 low pressure mercury lamps (254 nm) of 150 W each, incident light 70 W/m ² .	66-94	De la Cruz et al., 2013
UV/H ₂ O ₂	Pilot	RMW	5-7.5 ²	333 ng/L	20-50 mg H ₂ O ₂ /L. 5 low pressure mercury lamps (254 nm) of 150	82-99	De la Cruz et al., 2013

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						W each, incident light 70 W/m ² .		
sunlight/Ti O ₂ (CPC reactor)	Pilot	SRMW	13	100 µg/L	TiO ₂ immobilized on glass spheres.	50-80		Miranda-García et al. 2011
sunlight/Ti O ₂ (CPC reactor)	Pilot	RMW	15-50	56 ng/L	0.2 g TiO ₂ powder/L.	65-80		Bernabeu et al. 2011
RO	Pilot	Secondary treated wastewater	-	147 ng/L	Saehan 4040 FL, Flux = 20 L/(m ² .h)	>99		Snyder et al. 2007
		RMW/primary treated wastewater	7.8	181-410 ng/L	Saehan 4040 FL, Osmonics AK4040, Flux = 17-20 L/(m ² .h)	>99 (from 2 pilots)		
		Secondary/Tertiary treated wastewater	-	237-271 ng/L		>99 (from 2 pilots)		
					Hydranautics ESPA2			
RO	Pilot	RMW		64-99 ng/L	Ropur TR70-4021-HF	>99		Dolar et al. 2012
NF	Pilot	Effluent UWTP	-	640 ng/L	Flat sheet, Flux = 1-3 L/(m ² .h), TMP = 0.3-0.7 bar	12		Röhricht et al. 2009, 2010
NF	Pilot	RMW	-	300-380 ng/L	FILMTEC NF90-4040, 200 Da	78-92		Cartagena et al. 2013
RO					FILMTEC BW30-4040	82-93		
NF	Pilot	RMW	-	100-500 ng/L	Filmtec NF90 MWCO=200 Da, Flux = 18 L/(m ² .h)	79		Mamo et al. 2018
RO					Hydranautics ESPA2 MWCO 100 Da, Flux = 18 L/(m ² .h)	100		

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743

744 ¹RMW= real municipal wastewater; SRMW= spiked real municipal wastewater; ²TOC.

745

746 3. Multi-barrier approach for a safe treated wastewater reuse in agriculture

747 3.1 Treatment trains for a safe reuse

748 To make wastewater reuse safe for crop irrigation, a multi-barrier approach to wastewater
749 treatment is necessary. These barriers should include typical processes for urban wastewater
750 treatment (namely, primary mechanical pre-treatment, possible primary settling, biological
751 treatment etc.) and advanced treatments. Possible options of treatment trains (TTs) providing
752 different effluent qualities are presented in Figure 1.

753 As matter of fact, no specific regulation on CECs (except in Switzerland) and ARB&ARGs is
754 in force that can justify a prioritization for these contaminants with respect to more traditional
755 parameters (in particular bacteria indicators such as total coliforms and *E. coli*) regulated in
756 different countries and guidelines for wastewater reuse. In particular, as ARB are of concern,
757 total *E. coli* population was suggested to be a good indicator for the inactivation of the antibiotic
758 resistant fraction (Fiorentino et al., 2015).

759 The minimum treatment scheme for safe reuse should include a conventional depth filtration
760 downstream of a biological process (or an UF membrane as in case of MBR, Fig.1, b), followed
761 by a disinfection unit with UV radiation (Fig.1, a). This TT should effectively allow to address

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762 typical parameters (e.g., biochemical oxygen demand (BOD), chemical oxygen demand (COD),
763 total suspended solids (TSS), *E. coli* etc.) set in wastewater reuse regulation and guidelines.

764 Chemical disinfection (in particular by chlorine) (Fig.1, c) is cheaper compared to other
765 disinfection options but the formation of DBPs should be considered, and the TT may become
766 expensive compared to other options if DBPs are removed before reuse.

767 It has to be noted that, chemical disinfectants (such as chlorine and PAA) as well as an MBR
768 with UF membrane and UV radiation are poorly effective in the removal of CECs.

769

770

Figure 1 - see below

771

772 Therefore, if (i) the corresponding limit for bacterial indicators is so stringent that UV
773 disinfection is not sufficient and/or (ii) CECs contamination should be effectively minimized,
774 other, more effective treatment technologies need to be considered (Fig.1, d-g).

775 Among AOPs, ozonation and photochemical processes showed interesting results in the
776 removal of CECs and ARB. In particular, in the short term, ozonation and UV/H₂O₂ processes
777 are more attractive options (Fig.1, d) compared to other photo-driven AOPs to abate CECs as
778 well as to effectively inactivate bacteria (Rizzo et al., 2019a) because:

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- 779 1. their efficiency has been confirmed by different works available in scientific literature.
780 However, ozonation needs considerably less energy compared to UV/H₂O₂ treatment
781 for the same CEC abatement level and shows full-scale application;
- 782 2. other homogeneous photocatalytic processes (such as photo-Fenton) may request
783 additional costs (e.g., pH adjustment, chelating agents' addition) and/or have not yet
784 been exhaustively investigated (e.g., UV/free chlorine, UV/PAA, sulfate radical based
785 AOPs);
- 786 3. heterogeneous photocatalytic processes still have serious technological barriers for full-
787 scale application.

788 It is important to note that ozonation and AOPs typically ask for a biological post-treatment,
789 *i.e.* a biological sand or activated carbon filtration, to remove biodegradable oxidation by-
790 products and transformation products (Fig.1, d). Rapid depth filtration or alternatively a
791 dissolved air flotation treatment may be used as pre-treatment method just before AOP in the
792 event that residual suspended solids should interfere with subsequent processes.

793 Adsorption to GAC in packed reactors followed by UV disinfection (unlike O₃ and UV/H₂O₂,
794 adsorption is not a disinfection process) is another option to improve the quality of effluent
795 wastewater before reuse (Fig.1, e). In order to prevent GAC packed reactors from a fast
796 clogging and increase back flushing intervals, cloth or rapid sand filtration may be used to
797 remove suspended solids before the adsorption process.

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798 If PAC adsorption is used in combination with the biological process (by adding PAC into the
799 biological treatment) or as a separated unit thereafter, either depth filtration and/or MF/UF
800 membrane processes should be used to remove residual PAC particles before discharge
801 (Fig.1, f). As in GAC treatment, a UV disinfection may have to be installed.

802 Finally, membrane filtration with NF or RO followed by UV disinfection is another possible
803 option for advanced treatment of wastewater before reuse (Fig.1, g). Pre-treatment by sand
804 filtration can be used to remove suspended solids to control membrane fouling, although it is
805 more common to filter settled effluent directly with MF or UF membranes. MF and UF
806 membranes also provide suitable pre-treatment for the NF or RO step (in such a case final
807 disinfection by UV radiation is not necessary for crop irrigation). It is worthy to mention that
808 RO treatment would be additionally beneficial for crop irrigation because of the removal of
809 salts from the effluent. However, for membrane technologies to become sustainable there is
810 need for a deep study of the adequate treatment and/or disposal of concentrates on a case by
811 case basis. Implementation of effective concentrate treatment has the potential to enhance
812 treatment efficiency, move towards a near zero-liquid discharge and avoid unwanted discharge
813 of CEC.

814

815 3.2 Advantages, drawbacks and recommendations of the treatment schemes

816 The main objective of this discussion and analysis is to suggest the “best available technologies
817 able to minimize the release of microcontaminants including ARB&ARGs, and biological risk,

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818 and fulfill requirements for a safe reuse for crop irrigation”. Important issues for all TT
819 discussed before are summarized in Table 2. Accordingly, and considering that no exhaustive
820 comparative studies addressing CECs and ARB&ARGs removal by advanced treatment
821 methods are available in scientific literature (Rizzo et al., 2019a), a comparative economic
822 evaluation would be questionable. In particular, advanced treatment methods have been
823 compared in terms of either CECs removal, costs, disinfection efficiency, ARB and ARGs
824 removal, formation of DBPs and oxidation reaction products, and final toxicity, but the whole
825 impact on the environment through the simultaneous evaluation of all these issues has not been
826 investigated (Rizzo et al., 2019a). A recommendation needs to be case-specific, taking into
827 account possible regional regulations on wastewater reuse for crop irrigation, intake and
828 required water quality, and local climate conditions, and the relative importance of each aspect
829 needs to be carefully evaluated.

830

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Table 2 Advantages, drawbacks and recommendations for each TT in Figure 1.

TT (advanced treatment)	Advantages	Drawbacks	Recommendations
a or b (UV)	<ul style="list-style-type: none"> • Effective disinfection (including ARB inactivation) • No DBPs formation compared to chemical disinfection 	<ul style="list-style-type: none"> • If local standards for reuse are too stringent for residual bacterial density, UV may not be sufficient • Poor/no CECs removal • Partial removal of ARGs 	<ul style="list-style-type: none"> • Compliance with local residual bacterial density standards should be evaluated
c (chemical disinfection)	<ul style="list-style-type: none"> • Effective disinfection (including ARB inactivation) 	<ul style="list-style-type: none"> • Poor/no removal of CECs and ARGs • Formation of DBPs • If local standards for reuse are too stringent for DBPs, some disinfectant cannot be used (e.g., chlorine in Italy) 	<ul style="list-style-type: none"> • Toxicity tests recommended • DBPs (depending on the disinfectants used) should be monitored
d (O ₃ /AOP and biological post-treatment)	<ul style="list-style-type: none"> • Effective disinfection (including ARB inactivation) • CECs abatement high during ozonation and (solar) photo Fenton, moderate with UV/H₂O₂ • Full-scale evidence on practicability only for O₃ 	<ul style="list-style-type: none"> • Formation of some DBPs (NDMA, bromate) during ozonation • Formation of oxidation transformation products during AOP and ozonation • partial ARGs removal 	<ul style="list-style-type: none"> • Toxicity tests recommended • NDMA and bromate should be monitored in O₃ treatment

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e (GAC and UV)	<ul style="list-style-type: none"> • effective disinfection by UV • high CECs removal by GAC • full-scale evidence on practicability 	<ul style="list-style-type: none"> • Poor/no removal of ARB&ARGs by GAC alone • for UV see above, TT a & b 	<ul style="list-style-type: none"> • Decreasing adsorption capacity with increasing bed volume should be taken into account
f (PAC and UV)	<ul style="list-style-type: none"> • Effective disinfection by UV • High CECs removal by PAC • Full-scale evidence on practicability for CEC removal by PAC 	<ul style="list-style-type: none"> • Poor/no removal of ARB&ARGs by PAC alone • For UV see above, TT a & b 	
g (NF or RO membrane filtration, with potential pre-treatment with MF or UF membranes)	<ul style="list-style-type: none"> • Effective disinfection for bacteria (incl. ARB) and protozoa for all membranes; viruses well removed by UF, NF & RO • ARGs well removed by NF and RO • CECs removal from poor (MF, UF) to very good (NF, RO) depending on membrane type, • RO and partially also NF reduce salinity • For post UV-C see TT a & b 	<ul style="list-style-type: none"> • Poor/no removal of ARGs at full-scale by MF (for UF some removal is expected) • Poor CECs removal for MF and UF • High energy requirements for NF and RO • Generation of a substantial concentrate waste stream by NF and RO • For post UV-C see TT a&b 	<ul style="list-style-type: none"> • Impact of membrane characteristics on disinfection, ARB, ARG, and CEC removal should be carefully considered in design • Consider AOP instead of UV disinfection if the risk of unknowns and spills is considered high • Consider high UV doses if NDMA can be suspected in the membrane effluent (e.g. following prior chloramination)

832

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833 4. Concluding remarks

834 The safety of treated wastewater to be reused for crop irrigation is a relevant issue worldwide.
835 Recently the interest has increased at EU level and stimulated a discussion among policy
836 makers, scientists, professionals, practitioners and other stakeholders, because the European
837 Commission is about to approve a regulation on “Minimum requirements for water reuse”
838 (European Parliament, 2019). Accordingly, the aim of this paper is to provide a technical
839 contribution to this discussion by recommending possible advanced treatment options to make
840 wastewater reuse safer, in particular with regard to the removal of CECs and ARB&ARGs.
841 Different factors affect the choice of the most suitable treatment approach (i.e., water quality,
842 local regulation/restrictions, process costs, type of crop, irrigation method, soil type,
843 environmental footprint, social acceptance, etc.). Nevertheless, an attempt was made in this
844 manuscript by discussing possible BATs for the advanced treatment of urban wastewater
845 including their advantages and drawbacks.

846 The main conclusion of this work, that gathers the efforts of a group of international experts,
847 members of the NEREUS COST Action ES1403, is that a single advanced treatment method is
848 not sufficient to minimize the release of chemical CECs and ARB&ARGs and make wastewater
849 reuse for crop irrigation safer, but a smart combination of them (Figure 1) and a suitable
850 monitoring program (Table 2) would be necessary. This conclusion stems from the awareness
851 that each treatment method has its own weaknesses/drawbacks, for example:

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- 852 • a biological post-treatment to remove oxidation by-products may be necessary when
853 ozonation or AOP is used as advanced treatment.;
- 854 • ozonation and AOPs require toxicity monitoring because of possible formation of
855 problematic oxidation reaction products;
- 856 • adsorption processes should be followed by an effective disinfection process (i.e., UV
857 disinfection) to meet the stringent limits for wastewater reuse;
- 858 • if PAC is used, a subsequent filtration or membrane process should be applied to remove
859 the adsorbent particles;
- 860 • chemical disinfection is not effective in the removal of CECs and ARGs, thus it should
861 be coupled to other advanced treatment methods. Moreover, possible formation of DBPs
862 (i.e., chlorination by products) should be considered, and a subsequent treatment for
863 their removal may be necessary;
- 864 • NF or RO membrane technology would require a pre-treatment (i.e., sand filtration) to
865 prevent clogging and a sustainable solution for the management of membrane
866 concentrate.

867 Further comparative studies among different advanced treatment methods on real wastewater,
868 using different criteria (i.e., CECs removal, ARB&ARGs, toxicity, DBPs, costs) are
869 recommended. The results will be useful to UWWTPs managers to select the most suitable options
870 to be implemented at their own facilities to successfully address wastewater reuse challenges.

871

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872 **Acknowledgments**

873 The authors would like to acknowledge the COST Action ES1403 NEREUS “New and
874 emerging challenges and opportunities in wastewater reuse”, supported by COST (European
875 Cooperation in Science and Technology, www.cost.eu), for enabling the collaboration among
876 the authors of the paper.

877 Disclaimer: The content of this article is the authors' responsibility and neither COST nor any
878 person acting on its behalf is responsible for the use, which might be made of the information
879 contained in it.

880 LR acknowledges University of Salerno for the financial support through FARB-2017, Ref.
881 ORSA178411. WG acknowledges funding from the Economy and Knowledge Department of
882 the Catalan Government (Consolidated Research Group program: ICRA-TECH - 2017 SGR
883 1318) and from the Spanish Ministry of Science, Innovation and Universities, the State Agency
884 of Investigation and EU FEDER program (project INVEST: RTI2018-097471-B-C21). SM
885 wishes to thank the Spanish Ministry of Economy and Competitiveness for funding under the
886 ECOSAFEFARMING Project (International Joint Programming Actions, reference: PCIN-
887 2017-005) and 2016 Water and FACCE JPIs Joint Call. JASP acknowledges the Spanish
888 Ministry of Economy and Competitiveness and the European Regional Development Fund
889 (ERDF) for funding the project CTQ2016-78255-R. PK acknowledges the financial support
890 provided by NIVA's Strategic Institute Initiative “Urban water challenges” (Research Council
891 of Norway, contract no. 160016).

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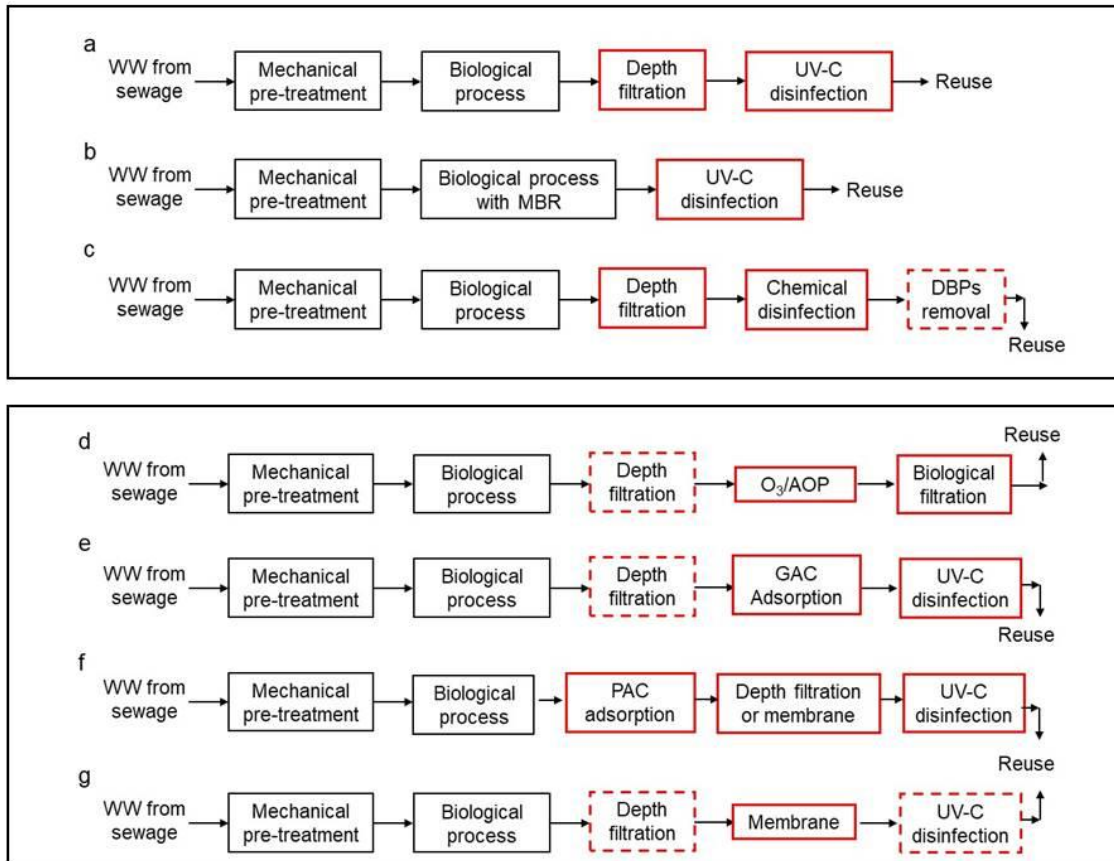
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1464

1465 **Figure 1.** Different options of treatment trains for urban wastewater reuse to address traditional
 1466 parameters set in wastewater reuse regulation and guidelines (e.g., BOD, COD, TSS, *E. coli*
 1467 etc.) (a, b, c) and to effectively remove CECs in addition to the typical parameters (d, e, f, g).
 1468 Advanced treatment in red lines; red dotted lines mean that process application should be
 1469 evaluated case by case. “Biological process” followed by “depth filtration” may be replaced by
 1470 “MBR” for treatment trains “d” and “e”.

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