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The best available technologies able to minimize the release of contaminants of emerging concern, including ARB&ARGs, and fulfil requirements for a safe reuse

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This article is based upon work from the COST Action ES1403: New and emerging challenges and opportunities in wastewater reuse (NEREUS), supported by COST (European Cooperation in Science and Technology).

Contents

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1. Introduction

The objective of this report is to discuss available technologies able to minimize the release of microcontaminants, including antibiotic-resistant bacteria and antibiotic resistance genes (ARB&ARGs), and biological risk, and fulfill requirements for a safe reuse of urban wastewater. This objective was addressed by reviewing the relevant scientific literature and by merging them with data not yet published, introduced by international experts (invited speakers) and discussed by the WG4 NEREUS members during the Action meetings, and particularly the one held in Patras (Greece) on March 22-23, 2017. It is noteworthy that literature review and consultation with experts revealed that defining best available technologies (BATs) is tricky as the performance of technologies will be very case specific, depending on the source effluent quality, the plurality of possible target qualities and the variety of possible reuse applications.

Possible technologies for advanced treatment and reuse of urban wastewater are introduced in the second chapter of this report. Specifically, ozonation, activated carbon adsorption, chemical oxidants/disinfectants, UV-C radiation, advanced oxidation processes and membranes are explained by evaluating their capability to effectively remove contaminants of emerging concern (CECs) and ARB&ARGs. In Chapter 3, possible treatment trains including the above discussed BATs are explained and the minimum treatment train for a safe reuse is suggested. Finally, possible advantages, drawbacks and recommendations of the proposed treatment trains are summarized.

2. Overview of available technologies for advanced treatment and reuse of urban wastewater

2.1 Ozonation

2.1.1 Removal of CECs

The oxidation capacity of the ozone process relies on the strong oxidation potential of both molecular ozone and HO radicals ($HO^{\bullet}$) (2.07 and 2.8 V against standard hydrogen electrode, respectively). While ozone reacts selectively with compounds containing electron-rich moieties (such as olefins, deprotonated amines or activated aromatics), HO• are characterized by low selectivity and a fast reaction with a wide range of organic and inorganic compounds (von Sonntag, 2007). Ozonation has been widely investigated in the removal of different CECs from urban wastewater since more than 10 years ago (Ternes et al., 2003). Hollender et al. (2009) investigated the removal efficiency for 220 micropollutants

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at a full-scale UWWTP upgraded with ozonation (followed by sand filtration). Compounds such as sulfamethoxazole, diclofenac, or carbamazepine were eliminated to concentrations below the detection limit (>85%)at an ozone dose of 0.47 g O_3 g^{-1} DOC. Compounds more refractory to oxidation by ozone, such as atenolol and benzotriazole, were eliminated at higher ozone dose (~0.6 g O_3 g-1 DOC). to the rate >85%. The high efficiency of ozonation in the removal of CECs from wastewater was also confirmed in subsequent studies (e.g., Antoniou et al. 2013; Magdeburg et al. 2014).

2.1.2 Effect on ARB&ARGs

Unlike CECs, the effect of ozonation process on ARB&ARGs has been poorly investigated so far. Ozone inactivation mechanisms include the disruption of bacterial cell wall (with release of cellular constituents), damages of nucleic acids (breaking aromatic structure), and breakage of carbon-nitrogen bonds of proteins leading to depolymerisation (Alexander et al., 2016). The inactivation efficiency by ozonation depends on wastewater characteristics (e.g., oxidant demand), ozone concentration, the target organism and contact time. In a culture-based study, antibiotic resistant strains of *Escherichia coli*, enterococci, and staphylococci were found to be more resistant towards ozonation compared to the respective antibiotic sensitive strains (Lüddeke et al., 2014). Zhuang et al. (2015) investigated the effect of ozonation on two ARGs (sul1 and tetG) and the integrase gene of class 1 integrons (intI1); a quite high ozone dose (177.6 mg/L) was necessary to achieve 1.68-2.55 log reductions of ARGs. Alexander et al. (2016) investigated the effect of ozonation on clinically relevant ARB and ARGs. Diverse patterns of resistances and susceptibilities of opportunistic bacteria and accumulations of some ARGs were observed after ozone treatment at 0.9 ± 0.1 g ozone per 1 g DOC in clarified wastewater. Ozone affected microorganisms in different ways, with a high susceptibility of enterococci (almost 99% reduction) compared to Pseudomonas aeruginosa, that displayed only minor changes in abundance after treatment. The investigated ARGs demonstrated an even more diverse pattern with 2 orders of magnitude reduction of erythromycin resistance gene (ermB) but a simultaneous increase in the abundance of ARGs (van_A, bla_{VIM}) within the surviving wastewater population.

2.1.3 Formation of oxidation by-products

Ozonation can result in the formation of biologically potent (e.g. toxic, mutagenic, etc.) oxidation by-products. Among them, N-nitrosodimethylamine (NDMA) and bromate are of particular concern for human health because they are carcinogenic. Therefore, NDMA and bromate need to be measured to test the feasibility of ozonation as an option for advanced wastewater at a specific location (Schindler Wildhaber et al. (2015). Only if their formation is

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low and below or in the range of the drinking water standards (e.g. 14 ng L^{-1} and <10 µg L^{-1}, Hollander et al., 2009), ozonation is suitable. Bromate results from the reaction of O_3 and $HO^{\bullet}$ with bromide. NDMA can be formed from the reaction of secondary amine precursors (e.g. hydrazines, sulfamide functional groups) with yields up to ≥ 50% (Kosaka et al. 2009; Schmidt and Brauch 2008; von Gunten et al. 2010; Krasner et al. 2013, Sgroi et al., 2014). Because precursors are mostly unknown or unidentified in wastewater, the formation of NDMA cannot be excluded a priori. NDMA can also already be present in the WWTP influent.

To minimize the release of biodegradable compounds including e.g. transformation by-products of CECs formed during ozonation biological sand filtration (or adsorption) is recommended to be used. For the evaluation of the water quality after ozonation, specific and unspecific toxicity of the treated wastewater needs to be measured with bioassays (Schindler Wildhaber et al. (2015).

2.1.4 Application at full scale as advanced treatment of urban wastewater

Ozonation is well established in drinking water treatment, but has only recently been applied at full scale as advanced treatment of urban wastewater in Europe for the removal of CECs before discharge into the environment. In particular, in Switzerland, ozonation is considered one of the best available technologies to meet the requirement of the new Swiss water protection Act (micropollutants removal by 80% relative to the raw wastewater; Eggen et al. 2014), which requires a WWTP upgrade within the next twenty years (so far two full-scale plant with ozonation are in operation). In Germany, in spite of the missing legislative requirements on CEC, the two federal states Baden-Württemberg (BW) and North Rhine-Westphalia (NRW) made the decision to upgrade several plants for advanced treatment by ozonation (or activated carbon). In Austria, pilot-scale experiments with ozonation (and subsequent biologically activated carbon filtration) are running with foreseen full-scale application in the near future for specific situations such as missing receiving surface water and subsequently ground water recharge. In France, the first full-scale application with ozonation was implemented in 2013 at the WWTP of St. Pourçain-sur-Sioule. The occurrence of organic matter (measured as dissolved organic carbon, DOC) and other readily oxidizable compounds (such as nitrite) in the effluent of biological treatment affect ozone consumption and should be taken into account when defining the ozone dose for the removal of CEC. A ozone dose in the range of 0.4 – 0.7 gO_3/gDOC (in absence of nitrite) was found to be suitable to efficiently remove micropollutants (Hollender et al. 2009, McArdell et al. 2015).

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2.2 Activated Carbon adsorption

2.2.1 Removal of CECs

Unlike oxidation/disinfection, adsorption is a separation process which does not result in the formation of problematic by-products. Activated carbon is the most used adsorbent in water treatment for the removal of organic and inorganic pollutants dissolved in water. Activated carbon has been widely investigated in the removal of CECs from wastewater (Grassi et al., 2013; Rizzo et al., 2015; Ahmed, 2017; Kovalova et al., 2013). Packed bed adsorption reactors with granular activated carbon (GAC) as adsorbent material are commonly used in drinking water treatment, but their application as advanced urban wastewater treatment at full scale is not so common due to process costs; its advantage compared to powder activated carbon (PAC) is based on the fact that it is easier to use, can be recovered and regenerated after use. PAC can be applied as a post-treatment or dosed into the biological unit in WWTPs and, due to its smaller particle size (higher specific surface area), is more efficient compared to GAC in the removal of water pollutants and specifically CECs (Nowotny et al., 2007).

2.2.2 Effect on ARB&ARGs

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

2.2.3 Application at full scale as advanced treatment of urban wastewater

Activated carbon adsorption has been recently applied at full scale as advanced treatment of urban wastewater as alternative to ozonation, particularly in Switzerland and Germany, for the removal of CECs before effluent discharge into the environment. Depending on DOC and operation technology, a dose of 10-20 mg/L PAC can be recommended (Böhler et al. 2012). In full-scale applications in Switzerland overall costs, including investment and operation, for PAC (0.10-0.15 CHF/m^3, 1 CHF being 0.9358 € on April 23, 2017, for dosing 10 mg/L in large plant with 590'000 p.e.) were found to be higher than for ozonation (0.04-0.06 CHF/m^3, for dosing 5 mg/L in a large plant) (Mc Ardell et al., 2015, Abegglen et al. 2012). Also in PAC treatment, a post-treatment is needed for separation of residual PAC material. Operation cost evaluation from 8 WWTPs in NRW (Germany), 3 with ozonation, confirmed that ozonation (0.05 €/m^3) is cheaper than activated carbon adsorption (0.085 and 0.065 €/m^3 for GAC and PAC, respectively) (Antakyali, 2017). Moreover, the use of GAC-packed reactors is more

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restricted since it does not allow to react to certain conditions (e.g. rainy periods), whereas PAC dose can be increased.

2.3 Chemical oxidants/disinfectants

Chlorination is by far the most common method of wastewater disinfection, but the concern for human health and the environment related to the formation of toxic by-products (e.g., trihalomethanes, haloacetic acids) is increasing the interest towards alternative chemical disinfectants, such as peracids. Among them, peracetic acid (PAA) already finds different applications at full scale in WWTPs (Formisano et al., 2016; Di Cesare et al., 2016). Accordingly, chlorination and PAA disinfection are discussed in the subsequent sub-paragraphs.

2.3.1 Chlorination

Wastewater disinfection by chlorine is typically performed by chlorine gas (in medium – large WWTPs) or hypochlorites (either calcium or sodium). Limited studies have focused on the removal of CECs by chlorine and removal efficiencies are quite poor, in particular if compared to oxidation/disinfection processes with higher oxidation potential such as ozone and other advanced oxidation processes. For example, Li and Zhang (2011) reported removal of antibiotics during wastewater treatment with chlorine in the range 18% (roxithromycin) – 40% (trimethoprim), but it is worthy to mention that cephalexin was removed by 99% and that chlorine dose is not reported.

Another important aspect to mention is that in effluents with incomplete nitrification, chlorine combines with ammonia to form chloramines or so-called combined chlorine. Chloramine chemistry is complex and will not be discussed further here, but it is noteworthy that chloramines are weaker oxidants and disinfectants compared to free chlorine. NDMA is a typical disinfection byproduct when chloramines are generated in wastewater effluents, hence if this is of concern NDMA formation must be monitored.

The effect of chlorination on ARB has been investigated since the 70's (Grabow et al., 1976). Although the chlorination process was found to effectively decrease antibiotic resistant *E. coli* in wastewater, it may select bacterial population by increasing antibiotic resistant *E. coli* strains compared to the corresponding total population (Fiorentino et al., 2015). Moreover, investigations on ARGs showed that they can survive the chlorination process. For example, ARGs *ere*A and *erm*B persisted in chlorinated (15 mg Cl_2 min L^{-1}) urban wastewater samples (Yuan et al. 2015) and chlorination was found to be effective in ARGs removal (3.16 Log for

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sulI and 3.24 Log for tetG after 120 min treatment) only at non-realistic chlorine concentration (160 mg L^{-1}) (Zhuang et al., 2015). Actually, these results are in agreement with previous investigations at full scale where (1) chlorination did not prove to have significant contribution to ARGs (tetw, tetO, sulI) and ARB reduction (Munir et al., 2011), (2) nor affected tet and sul genes (Gao et al., 2012).

On the contrary sub-inhibitory concentrations (lower than minimum inhibitory concentrations [MICs]), namely 0.1-1 mg L^{-1} Cl_2 for free chlorine, led to concentration-dependent increases in intra-genera (*E. coli*) conjugative transfer by 3.4–6.4 in drinking water (Zhang et al., 2017). Moreover, the inter-genera (*E. coli* to *Salmonella typhimurium*) conjugative frequencies were slightly increased compared with controls.

2.3.2 Peracetic acid

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

In spite of no significant formation of DBPs resulting from wastewater disinfection by PAA when low doses are used (<5-10 mg/L) (Nurizzo et al., 2005), PAA was found to be toxic for bacteria and crustaceans, even at concentrations lower than the ones commonly used in wastewater disinfection (2-5 mg/L). But when PAA was compared to other disinfection processes, a lower toxicity against aquatic organisms was observed. In particular da Costa et al. (2014) compared PAA, UV light, ozone, and sodium hypochlorite against *Ceriodaphnia silvestrii*, *Daphnia similis*, *Chironomus xanthus*, and *Danio rerio* and toxicities were in the order of free chlorine > ozone > UV > PAA.

Due to its lower oxidation potential compared to ozone and hydroxyl radicals, PAA has not been investigated for the removal of CECs from wastewater, to our knowledge, and only a few works evaluated the effect on ARB&ARGs. Huang et al. (2013) evaluated the effect of PAA on the inactivation and regrowth of ARB after exposure to 20 mg PAA L^{-1} for 10 min in reclaimed water. Inactivation was higher for ampicillin-resistant bacteria (2.3-log) than for total heterotrophic bacteria (2.0-log) and tetracycline resistant bacteria (1.1-log). Moreover, the regrowth of chloramphenicol-and tetracycline-resistant bacteria, as well as total heterotrophic bacteria was more than 10 fold compared to those in the untreated wastewater sample (22 h stilling culture after exposure to 2 or 5 mg/L PAA as for 10 min). Di Cesare et

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al. (2016) evaluated the fate of diverse ARGs, heavy metal resistant genes and of a mobile element (the class I integron) in three WWTPs using different disinfection processes. In 2 (*sul*II and *tet*A) out of 4 (*erm*B and *qnr*S) of the quantified ARGs, a decrease was observed after PAA treatment.

2.4 UV-C radiation

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

2.4.1 Removal of CECs

UV-C radiation is not at all or poorly effective in the removal of most of CECs from water and wastewater, but it can remove some antibiotics and other CECs at very high UV doses (Kim et al., 2009). For example, an almost complete removal of tetracyclines and ciprofloxacin was achieved but only at high UV doses (11,000 - 30,000 mJ/cm^2) (Yuan et al., 2011) and high removal efficiencies (86-100%) were also observed for sulfonamides (sulfamethoxazole and sulfadimethoxine) and quinolones (norfloxacin and nalidixic acid) (Kim et al., 2009).

2.4.2 Effect on ARB&ARGs

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

Efficient removal of heterotrophic bacteria harbouring resistance to erythromycin and tetracycline (equivalent log reduction being 1.4 and 1.1, respectively) at a UV dose of 5 mJ cm^{-2} were observed (Guo et al., 2013); as UV dose was increased (i.e. 20 and 50 mJ cm^{-2}), ARB were below the detection limit (1 CFU mL^{-1}), with tetracycline-resistant bacteria more resistant to UV radiation than those carrying resistance to erythromycin. Rizzo et al. (2013b)

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observed a total inactivation of a multi-drug resistant (amoxicillin, ciprofloxacin and sulfamethoxazole) *E. coli* strain (selected from a wastewater sample taken from the effluent of the biological process of a WWTP) after 60 min of UV irradiation (1.25×10^4 mJ cm^{-2}).

The effect of UV disinfection on ARGs is affected by UV dose too. UV-C doses ranging from 200 to 400 mJ cm^{-2} (at least one order of magnitude higher than those for the inactivation of host bacterial cells) were required to remove 3 or 4 Log units of ARGs, namely *amp*C, *mec*A, *tet*A and *van*A (McKinney and Pruden, 2012). Actually, also lower UV-C doses (5 -10 mJ cm^{-2}) were found to be effective in the removal of ARGs (namely *ere*A, *ere*B, *erm*A, *erm*B, *tet*A, *tet*O) but starting from lower initial ARGs copies mL^{-1} (Guo et al., 2013). The relative abundance of selected ARGs increased during ozonation and with low doses of UV and chlorine (Zhuang et al., 2015).

In full-scale monitoring (5 UWWTPs in the USA), UV radiation (and chlorine) did not prove to have significant contribution to ARGs (*tet*w, *tet*O, *sul*I) and ARB reduction (Munir et al., 2011). The statistical t-test between concentrations of ARGs abundance in pre- and post-disinfected effluent did not show any significant difference (t-test, $p>0.05$) between UV and chlorination disinfection processes. These results were confirmed in a subsequent study at full scale, where no significant difference in ARGs (namely, *erm*B, *qnr*S and *tet*A) was observed before and after UV disinfection, but for *sul*II (an increase was observed after disinfection) (Di Cesare et al., 2016).

2.5 Advanced oxidation processes

Advanced oxidation processes (AOPs) relies on the formation of reactive oxygen species (ROS), mainly hydroxyl radicals, that can remove a wide range of CECs (Rizzo, 2011) as well as inactivate microorganisms (Dunlop et al., 2010; Fiorentino et al., 2015). A possible classification of AOPs includes two groups: homogeneous processes (e.g., UV/H_2O_2, $UV/Fe/H_2O_2$, O_3, O_3/H_2O_2 etc.) and heterogeneous (solid semiconductors + light source, e.g., UV/TiO_2) photocatalytic processes. Homogeneous processes have been widely investigated as advanced treatment of urban wastewater and either are already applied at full scale (e.g., O_3) or are characterized by short-/mid-term perspective application (e.g., UV/H_2O_2, $UV/Fe/H_2O_2$) compared to heterogeneous photocatalytic processes. The main reason why heterogeneous photocatalytic processes are not ready for full-scale application as advanced urban wastewater treatment are related to photocatalyst preparation costs and reactor configuration. In particular, heterogeneous photocatalytic processes can be operated under two main configurations: (i) with the photocatalyst suspended in the reactor (i.e., slurry

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system) or attached to a support (i.e. immobilized system). Due to the higher specific surface area available, a slurry system is more effective than an immobilized one, but a subsequent separation process (e.g., coagulation, filtration, membrane) is necessary to recover the photocatalyst before effluent disposal or reuse. Some homogeneous photo processes (Solar/H_2O_2 or Solar/Fe/H_2O_2) can also be operated under natural sunlight thus saving energy costs (Klamerth et al., 2010;Ferro et al., 2015) and this can be considered as an attractive option for small WWTPs.

2.5.1 Removal of CECs

Due to their high redox potential HO radicals oxidize a wide spectrum of organic contaminants, eventually, at very high doses of oxidants, mineralizing them (by converting in salts, carbon dioxide and water); accordingly, AOPs successfully degrade several organic micropollutants (Klavarioti et al, 2009; Rizzo, 2011). These processes use oxidants like H_2O_2 or O_3 in combination with radiation, other oxidants or heterogeneous catalysts to form ROS. The most common AOPs studied are UV/H_2O_2, O_3/H_2O_2, O_3/UV, Fenton (Fe/H_2O_2), photo-Fenton (UV/Fe/H_2O_2) and heterogeneous photocatalysis (e.g., UV/TiO_2, UV/ZnO). Fenton and photo-Fenton processes are typically effective under acidic conditions (pH 3), but have been also successfully investigated under mild conditions (pH 5-6) and solar radiation in the removal of CECs from urban wastewater (Klamerth et al., 2010). The operation of (solar driven) photo-Fenton process under mild conditions, for the removal of CECs from urban wastewater allow to effectively decrease CECs also under low Fe (< 5 mg L^{-1}) and H_2O_2 (< 20 mg L^{-1}) concentrations, thus making the final separation of soluble iron species from the treated wastewater not necessary. Unlike photo-Fenton, solar-UV/H_2O_2 process can be operated at neutral pH, can successfully remove CECs, but needs longer reaction time compared to photo-Fenton (Ferro et al., 2015).

In heterogeneous photocatalysis the load of the catalyst may be a limiting factor. As a matter of fact, a low catalyst load can result in an insufficient production of hydroxyl radicals, whereas a high dose (above the optimum one) can reduce the transmittance of the UV light due to the increased turbidity (Rizzo, 2009). The presence of organic matter in urban wastewater can inhibit the degradation of CECs through the antagonistic adsorption reactions onto the catalyst surface. Accordingly, the optimum catalyst dose and contact time for the degradation of CECs are therefore water-specific. Other effects that may reduce CECs removal include: (i) scavenging of $HO^{\bullet}$ by anions (e.g., bicarbonates, chlorides, sulfates), forming radicals with lower oxidation potentials; (ii) screening effect, when light

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absorption by matrix components occurs at same wavelengths as the catalysts; (iii) turbidity and suspended solids that reduce light transmission through the water (Rizzo, 2009).

An estimation of energy costs was given recently for the 3 processes conventional ozonation, O_3/H_2O_2 and UV/H_2O_2 (Katsoyiannis et al. 2011). O_3/H_2O_2 was found to be more energy demanding (by 23%) for the abatement of an ozone-refractory compound than the conventional ozonation, due to the demand for H_2O_2 production. The UV/H_2O_2 process was shown to require significantly more energy than ozone-based processes by a factor of 3.5 - 36.5, depending on the water matrix and the path length. However, it is worthy to mention that the processes were compared in the degradation of only three organic micropollutants (namely atrazine, sulfamethoxazole and NDMA) and no toxicity tests were performed.

2.5.2 Effect on ARB&ARGs

AOPs can successfully inactivate ARB in urban wastewater (Karaolia et al., 2014; Rizzo et al., 2014a; Fiorentino et al., 2015), but the release of mobile genetic elements from bacterial cells, that may take place after disinfection process, and the potential to transfer antibiotic resistance through horizontal mechanism, have been poorly investigated. As previously discussed, conventional disinfection processes (namely chlorination and UV radiation) may not be successful in ARGs removal, at least with realistic doses of disinfectants or UV radiation (Guo et al., 2013; McKinney and Pruden, 2012; Munir et al., 2011; Yuan et al., 2015; Zhuang et al., 2015). Therefore, to evaluate if AOPs can be more effective than conventional disinfection processes in the removal of ARGs, Ferro et al. (2016) investigated the effect of UV/H_2O_2, under realistic conditions for wastewater treatment (natural pH (7.6) and 20 H_2O_2 mg L^{-1}), on antibiotic resistance transfer potential in urban wastewater. Despite the investigated process resulted in bacterial inactivation and a decrease of ARGs in intracellular DNA after 60 min treatment, UV/H_2O_2 process was not effective in ARGs removal from water suspension. Actually, an increase up to 3.7×10^3 copies mL^{-1} ($p > 0.05$) of *bla*$_{TEM}$ gene was observed in total DNA after 240 min treatment, while no difference ($p > 0.05$) was found for *qnr*S gene between the initial (5.1×10^4 copies mL^{-1}) and the final sample (4.3×10^4 copies mL^{-1}). Differently, Zhang et al. (2016) showed that UV/H_2O_2 can effectively remove ARGs (2.8-3.5 logs removal of *sul*1, *tet*X, and *tet*G, within 30 min treatment) but only under not feasible conditions in full scale plants (pH 3.5 and 340 mg H_2O_2 L^{-1}).

The effect of heterogeneous photocatalysis with TiO_2 on ARB&ARGs has been investigated in slurry and immobilized systems. Tsai et al. (2010) investigated the effect of UV-A/TiO_2 on

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three ARB in suspended system (methicillin-resistant Staphylococcus aureus (MRSA), multidrug-resistant Acinetobacter baumannii (MDRAB) and vancomycin-resistant *Enterococcus faecalis* (VRE)). MRSA was found to be as susceptible to photocatalytic process as the control organism *S. aureus*, while the susceptibility of MDRAB was double that of the control organism *A. baumannii* ($P < 0.05$). Finally, the susceptibility of control organism *E. faecalis* was 2.4 times that of VRE ($P < 0.05$).

Rizzo et al. (2014a) investigated the effect of different light sources and TiO_2 loadings (from 0.05 to 2.00 g TiO_2 L^{-1}) on the photocatalytic inactivation of an antibiotic resistant *E. coli* strain selected from an urban WWTP effluent. The optimum photocatalyst loading estimated by radiation absorption-scattering modeling was found to be 0.1 g TiO_2 L^{-1} for all lamps. Comparative kinetic tests between solar and solar simulated photocatalytic (SSP) processes using 0.05 g TiO_2 L^{-1} in suspension showed a quite similar inactivation behavior up to 30 min of irradiation, but only the SSP process resulted in a total inactivation of bacteria after 60 min of exposure. Rizzo et al. (2014b) also investigated the effect on AR *E. coli* strain inactivation of N-doped TiO_2 (NDT) photocatalysis compared with commercially available TiO_2 powders (namely Millennium PC50 and PC100), under solar simulated radiation (250 W lamp) and different photocatalysts loadings (0.025-0.5 g L^{-1}). The higher inactivation rate (8.5×10^5 CFU 100 mL^{-1} min^{-1}, after 10 min of irradiation) was observed for NDT photocatalyst at 0.2 g L^{-1} dose. Kinetic test at the optimum photocatalyst loading showed that total inactivation can be achieved after 60 min of irradiation. Finally, Dunlop et al. (2015) reported the effect of photocatalysis on the potential to induce ARGs transfer within sublethally injured ARB, by cultivation-based methods. The effect of photocatalytic treatment was evaluated on three strains of *E. coli*, an antibiotic sensitive strain (K12) and two antibiotic resistant strains (J-53R (rifampicin resistant) and HT-99 (chloramphenicol resistant), within an immobilized TiO_2 stirred tank reactor. Viable cell numbers (CFU mL^{-1}) of both ARB declined from 3 $\log_{10}$ to 0.5 $\log_{10}$ with 180 min PCD treatment in deionized water. However, subsequent recovery to 3 $\log_{10}$ of both ARB was observed during post treatment incubation at 37◦C for 24 h. No *E. coli* K12 were recovered immediately after 150 min treatment, or after post treatment incubation. These observations suggest that the ARB are less sensitive to the oxidative stresses involved in PCD treatment than the antibiotic sensitive strain. Four-fold greater gene pair conjugant numbers than in the (no treatment) control experiments were observed in PCD treated mixtures of J-53R and HT-99 cells (a 9:1 ratio). Both surviving bacterial cell numbers and conjugant pair numbers were lower when ARB were PCD treated in final effluent from an urban wastewater treatment plant. Accordingly, PCD treatment should be applied for enough

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time to avoid post treatment recovery from sub-lethal injury and the highly undesirable transfer of antibiotic resistant genes amongst bacteria during wastewater treatment.

2.6 Membranes

Membrane separation processes include microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO). NF and RO are effective in the removal of both organic and inorganic CECs (Bellona et al., 2004; Alturki et al., 2010; Garcia et al., 2013) and MF or UF are typically used as pre-treatment of either NF or RO to control membrane fouling. Besides CEC removal and disinfection provided (see subsequent sections), NF and specifically RO provide the opportunity to reduce the electrical conductivity of the effluent, which can be necessary depending on the downstream application of the treated effluent. However, a waste stream containing the separated salts and other pollutant is generated as well.

2.6.1 Removal of CECs

Removal of CECs by membrane processes is primarily based on size exclusion, although electrostatic interactions between charged solutes and negatively charged membranes also typically have an important role for charged solutes (Bellona et al., 2004). Hydrophobic trace contaminants have been shown to absorb to membrane surfaces reducing the rejection of these contaminants through both RO and NF, but has been shown to be particularly important when considering NF processes. A number of other factors typically also affect the removal of the target CECs (such as phenolic aromatic compounds) by membrane processes (Bellona et al., 2004). Depending on the type of membrane, the range of rejections of CECs by both RO and NF is quite large, but the rejection can be higher than 99% for high rejection RO membranes. However, in these membrane processes the CECs are accumulating in the rejected concentrate. The discharge of the concentrate to the environment can be problematic, as the original salt and pollutant freight of the secondary effluent, while not having increased in absolute mass, is now concentrated typically by a factor of 3 to 7, depending on the permeate water recovery percentage of the membrane process. The presence of the contamination in concentrated form can in fact be an opportunity for treatment.

Full-scale applications of RO technology are reported in potable reuse treatment trains, e.g. the Orange County Groundwater Replenishment System (California, USA), NEWater facilities at the Bedok, Kranji, Ulu Pandan and Changi facilities in Singapore and the Torreele Reuse Facility in Belgium (Raffin et al., 2013; Gerrity et al. 2013). RO is also used in direct potable reuse treatment trains, along with MF or UF, in Windhock in Namibia, Cloudcroft

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(New Mexico) and Big Spring (Texas) in USA (Gerrity et al. 2013). NF typically removes CECs in the 300 - 1000 molecular weight (MW) range, rejecting selected salts and most organic constituents and microorganisms, operating at higher recovery rates and lower pressures than RO processes. Accordingly, and when feasible, NF can be used instead of RO to save some energy, chemical and concentrate disposal costs (Yangali-Quintanilla et al., 2010). On the drawback of very high removal efficiencies for CECs, these technologies exhibit high energy consumption.

2.6.2 Effect on ARB&ARGs

As the separation principle is purely based on size, the removal of ARB&ARGs can be expected to behave very similarly to microbes not presenting antibiotic resistance. Microfiltration and ultrafiltration are commonly applied barriers for pathogens, with microfiltration being very effective against protozoa and bacteria, while due to a larger pore size, it is not very effective in removing viruses. Ultrafiltration removes all three classes of pathogens to a very high extent (2 to 4 log removal values (LRV)) (Hai et al. 2014).

NF and RO membranes present in theory an even smaller pore size and should be “perfect filters”. In fact, even for viruses’ removal > 6 LRV have been observed at pilot-scale. However, due to the modular engineering approach finding appropriate surrogate measurements remains a challenge to ensure disinfection during operation, at least at levels beyond e.g. the removal of electrical conductivity (Pype et al, 2016).

The effect of membrane filtration, in particular NF and RO, on ARB&ARGs, thus far, has been little discussed in the literature as the existing studies have focused mostly on MBRs and MF and UF membranes (Munir et al. 2011, Riquelme Breazeal et al. 2013, Rizzo et al. 2013, Yang et al. 2013, Du et al. 2014, Wang et al. 2015, Zhang et al. 2015, Sun et al. 2016, Threedeach et al. 2016).

As previously mentioned, membranes are able to remove bacteria due to membrane retention, thus contributing to reducing the spread of multiple antibiotic resistant strains (Verlicchi et al. 2015). For example, filtration of ARGs spiked WWTP effluent through the 100, 10 and 1 kDa membranes in the lab-scale stirred ultrafiltration cell reduced *vanA* and bla_{TEM} ARGs by 0.9, 3.5 and 4.2 log, respectively (Riquelme Breazeal et al. 2013). The removal of plasmid-associated antibiotic resistance genes improved further at the presence of colloidal material in the water matrix and the colloids influence became more apparent as the membrane pore size decreased. The DNA removal was attributed to membrane retention

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and following mechanisms: i) size exclusion of the DNA, ii) size exclusion of DNA-colloid complexes, or iii) interactions with the membrane material (Riquelme Breazeal et al. 2013).

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

To the authors knowledge, there are no studies investigating the effects of NF or RO membrane filtration on ARB&ARGs. However, according to Böckelmann et al. (2009) combination of UF and RO proved to be efficient barrier for the elimination of ARGs. ARGs *tetO* and *ermB* detected in WWTP effluent at concentrations of $1.05x10^7$ $\pm 3.54x10^6$ gene copies and $1.92x10^5$ $\pm 1.06x10^4$ gene copies respectively, were removed during the UF-RO process applied in the Torreele Reuse Facility. Noteworthy, *tetO* were detected again, at low concentrations, in subsequent sampling points: in the infiltration water before transport ($5.92x10^3$ ± 1.39 $x10^3$ gene copies) and in the groundwater after infiltration ($3.13x10^3$ $\pm 1.52x10^3$ gene copies).

3. Multi-barrier approach for a safe water reuse

3.1 Minimum treatment train for a safe reuse

To make wastewater reuse safe, a multi-barrier approach to wastewater treatment is necessary. These barriers should include typical processes for urban wastewater treatment (namely, primary mechanical pre-treatment (such as, grill treatment and grit removal), possible primary settling, biological treatment (e.g., either activated sludge, moving bed biofilm reactor (MBBR), membrane biological reactor (MBR) etc.) and advanced treatments.

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According to the scientific literature discussed in the previous paragraphs, the following statements can be made:

1. Ozonation can effectively remove CECs, but the possible formation of biologically potent oxidation by-products (e.g., NDMA and bromate) requires an evaluation and a subsequent biological sand filtration or adsorption unit;
2. AOPs can effectively remove CECs, but the formation of toxic oxidation products cannot be excluded;
3. Adsorption to activated carbon is effective in the removal of CECs, but cannot effectively inactivate bacteria, thus a subsequent disinfection unit is necessary;
4. Consolidated disinfection processes (namely, chlorine, UV radiation) can decrease ARB but cannot remove ARGs or CECs under conditions (disinfectant dose) feasible at full scale.

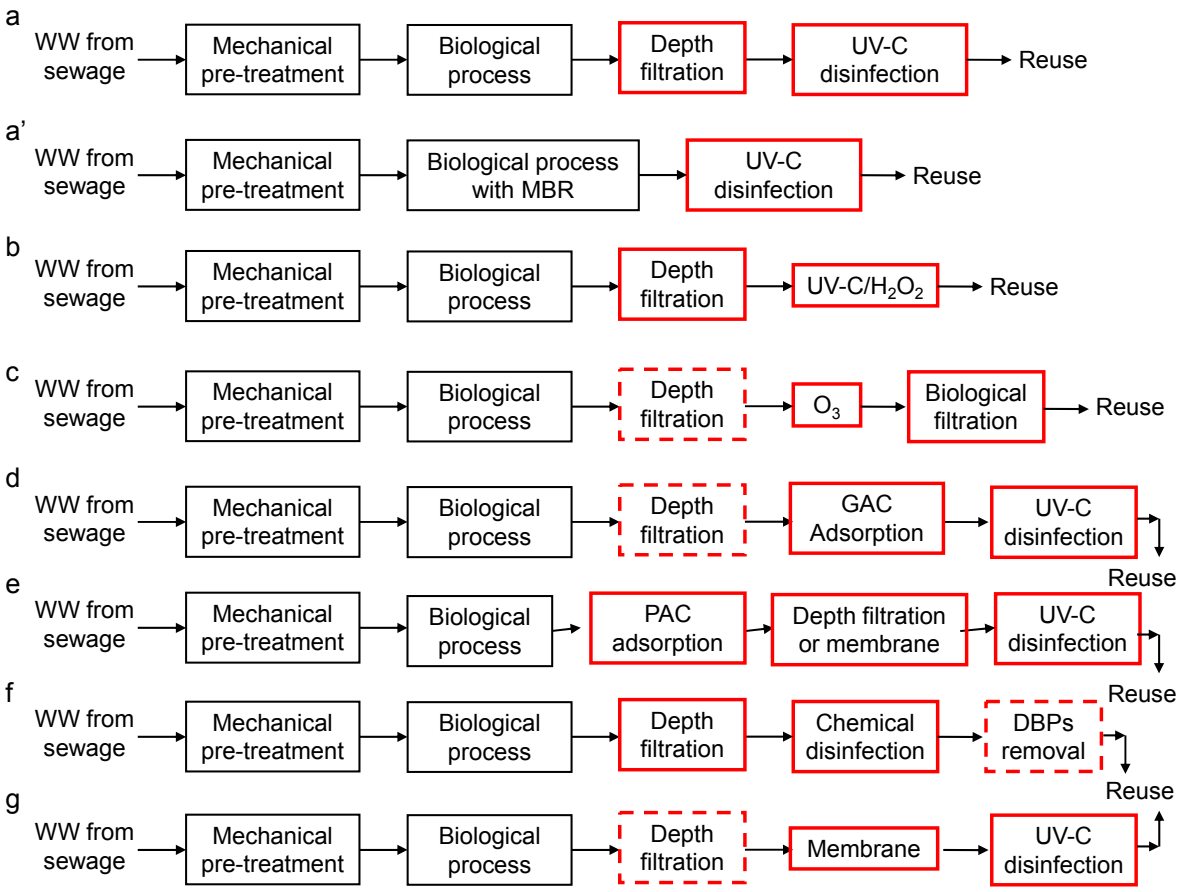

Figure 1 - Different options of treatment trains for urban wastewater reuse: advanced treatment in red lines; red dotted lines mean that process application should be evaluated case by case. According to "a" and "a'" treatment trains, additional options can be achieved by replacing "biological process" with "MBR" for treatment trains from "b" to "f" and by removing depth filtration.

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No specific regulation on CECs (except in Switzerland) and ARB&ARGs is in force that can justify a prioritization for these contaminants with respect to other more common contaminants (in particular bacteria indicators such as total coliforms and *E. coli*) regulated in different countries and guidelines for wastewater reuse.

The minimum treatment scheme for safe reuse should include a conventional depth filtration downstream of a biological process (not necessary only in case of MBR, fig.1a'), followed by a disinfection unit with UV-C radiation (fig.1a). This treatment train (TT) should effectively allow to address typical parameters (e.g., BOD, COD, TSS, *E. coli* etc.) set in wastewater reuse regulation and guidelines.

If (i) the corresponding limit for bacterial indicators is so stringent that UV-C disinfection is not sufficient and (ii) CECs contamination should be minimized, other TTs need to be considered (fig.1 b-g).

3.2 Other multi-barrier treatment trains

If the minimum TT (fig.1a, a') is not sufficient to meet wastewater reuse limits, and particularly a higher disinfection efficiency and a higher CECs removal efficiency are requested, other advanced TT options are possible (fig.1b-g).

Among AOPs, photocatalytic processes showed interesting results in the removal of CECs and ARB. In particular, UV-C/H_2O_2 process is recommended as best option (fig.1b) compared to other photo driven AOPs to remove CECs as well as to effectively inactivate bacteria because:

1. Its efficiency has been confirmed by different works available in scientific literature;
2. Other homogeneous photocatalytic processes (such as photo-Fenton) may request additional costs (e.g., pH adjustment);
3. Technologies for heterogeneous photocatalytic processes are not yet mature for full scale application, making the process more expensive than UV-C/H_2O_2.

Advanced treatment by ozonation typically asks for a post-treatment by biological filtration to remove oxidation by-products and biodegradable organic matter that may promote bacterial regrowth (fig.1c). Rapid depth filtration or alternatively a dissolved air flotation treatment may be used as pre-treatment method just before ozonation to remove residual suspended solids as well as to further decrease oxidant demand before ozonation (thus reducing O_3 dose).

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Adsorption by GAC in packed reactors followed by UV-C disinfection (unlike O_3 and UV/H_2O_2, adsorption is not a disinfection process) is another option to improve the quality of effluent wastewater before reuse (fig.1d). In order to prevent GAC packed reactors from a fast clogging, rapid sand filtration may be used to remove suspended solids before adsorption process.

If PAC adsorption is used in combination with biological process (by adding PAC in the aeration tank) or as separated unit after biological process, either depth filtration and/or membrane processes should be used to remove PAC before wastewater reuse (fig.1e).

Chemical disinfection (in particular by chlorine) (fig.1f) is cheaper compared to other disinfections options but the formation of DBPs should be taken into account, and the TT may be quite expensive compared to other options if DBPs should be removed before reuse.

Finally, membrane followed by UV-C disinfection is another possible option for advanced treatment of wastewater before reuse (fig.1g). Pre-treatment by sand filtration can be used to remove suspended solids to control membrane fouling, although it is more common to filter settled effluent directly with MF or UF membranes. MF and UF membranes also provide suitable pre-treatment for a possible sub-sequent NF or RO step.

3.3 Advantages, drawbacks and recommendations of the treatment schemes

In this final paragraph, the advantages and drawbacks of advanced treatment methods and TTs discussed and explained in the previous paragraphs are summarized (Table 1). Additionally, where relevant, recommendations are provided (Table 1).

The main objective of this report is to discuss “the best available technologies able to minimize the release of microcontaminants including ARB&ARGs, and biological risk, and fulfill requirements for a safe reuse“. Accordingly, and considering that no exhaustive comparative studies addressing CECs and ARB&ARGs removal by advanced treatment methods are available in scientific literature, a comparative economic evaluation would be questionable. In particular, advanced treatment methods have been compared in terms of either CEC removal, costs, disinfection efficiency, formation of DBPs and oxidation intermediates, and final toxicity, but the whole impact on the environment through the simultaneous evaluation of all these issues has not been investigated. A recommendation needs to be based case-specific on the reuse objective, taking particular country regulations, intake and required water quality, and local conditions into consideration, and the relative importance of each aspect needs to be carefully evaluated.

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Table 1 - Advantages, drawbacks and recommendations for each TT in fig.1

TT	Advantages	Drawbacks	Recommendations
a	• Effective disinfection • No DBPs formation	• If local standards for reuse are too stringent for residual bacterial density, UV-C may not be sufficient • Poor CECs removal • Possible formation of transformation products due to photolysis of organic contaminants • Poor/no removal of ARB&ARGs at full scale	• To check if residual bacterial density meets local standards
a'	• Effective disinfection • No DBPs formation	• If local standards for reuse are too stringent for residual bacterial density, UV-C may not be sufficient • Poor CECs removal • Possible formation of transformation products due to photolysis of organic contaminants • Poor/no removal of ARB&ARGs at full scale	• To check if residual bacterial density meets local standards
b	• Effective disinfection • Moderate-good CECs removal at lab/pilot scale • Good ARB removal at lab/pilot scale	• Possible formation of oxidation intermediates after UV/H_2O_2 process • No full-scale evidences on CECs and ARB&ARGs removal by	• Toxicity tests recommended

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		UV/H_2O_2	
c	Combination of O_3 and BF processes results in: • effective disinfection • high CECs removal • full scale evidence on practicability	• Formation of some DBPs (NDMA, bromate) after O_3 • Formation of oxidation intermediates after O_3 • High energy demand from ozone production No full scale evidences on ARB&ARGs removal by O_3	• Toxicity tests recommended • NDMA and bromate should be monitored
d	Combination of DF, GAC and UV-C processes results in: • effective disinfection • high CECs removal • full scale evidence on practicability	• Poor/no removal of ARB&ARGs at full scale by UV-C • Production of GAC needs high energy • Still under investigation if more activated carbon is needed compared to PAC • Less flexible in operation than PAC and ozonation to react to changes in wastewater composition • Adsorption capacity may change with each batch • Possible formation of transformation products due to photolysis of organic contaminants (lower than a and a' options due to the	• To check if residual bacterial density meets local standards • Test with different GAC products recommended

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		presence of adsorption process in the TT)	
e	Combination of AS+PAC, DF or UF, and UV-C processes results in: • effective disinfection • high CECs removal • full scale evidence on practicability for CEC removal by activated carbon	• PAC must be disposed • production of PAC needs high energy • adsorption capacity may change with each batch • Poor/no removal of ARB&ARGs at full scale by UV-C • Possible formation of transformation products due to photolysis of organic contaminants (lower than a and a' options due to the presence of adsorption process in the TT)	• To check if residual bacterial density meets local standards • Test with different PAC products recommended
f	• effective disinfection	• Poor/no removal of CECs and ARB&ARGs at full scale • Formation of DBPs • If local standards for reuse are too stringent for DBPs, some disinfectant cannot be used (e.g., chlorine in Italy)	• Toxicity tests recommended • DBPs (depending on the disinfectant used) should be monitored
g	• effective disinfection • depending on membrane type, from poor (UF) to high (NF, RO) CECs removal	• Poor CECs removal for UF • High energy requirements for NF and RO	• To check if residual bacterial density meets local standards in TT implementing UF

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	• can reduce salinity	• Poor/no removal of ARB&ARGs at full scale (for NF and RO an higher efficiency can be expected) • Possible formation of transformation products due to photolysis of organic contaminants	

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