Removal of micropollutants in WWTP effluent by biological assisted membrane carbon filtration (BioMAC)

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Removal of micropollutants and reduction of biological activity in a full scale reclamation plant using ozonation and activated carbon filtration

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ABSTRACT

Pharmaceutical compounds are found in secondary treated effluents up to $\mu g\,L^{-1}$ levels and therefore discharged into surface waters. Since the long term effects of these compounds on the environment and human health are, to date, largely unknown, implementation of advanced treatment of wastewaters is envisaged to reduce their discharge. This is of particular relevance where surface waters are used as drinking water sources and when considering indirect potable reuse. This study aimed at assessing the removal of organic micropollutants and the concurrent reduction of their biological activity in a full scale reclamation plant treating secondary effluent. The treatment consists of 6 stages: denitrification, pre-ozonation, coagulation/flocculation/dissolved air flotation and filtration (DAFF), main ozonation, activated carbon filtration and final ozonation for disinfection. For that purpose, representative 24-hour composite samples were collected after each stage. The occurrence of 85 compounds was monitored by LC/MS-MS. A battery of 6 bioassays was also used as a complementary tool to evaluate non-specific toxicity and 5 specific toxic modes of action. Results show that, among the 54 micropollutants quantified in the influent water, 50 were removed to below their limit of quantification representing more than 90% of concentration reduction. Biological activity was reduced, depending on the specific response that was assessed, from a minimum of 62% (AhR response) to more than 99% (estrogenicity). The key processes responsible for the plant's performances were the coagulation/flocculation/DAFF, main ozonation and activated carbon filtration. The effect of these 3 processes varied from one compound or bioassay to another but their combination was almost totally responsible for the overall observed reduction. Bioassays yielded complementary information, e.g. estrogenic compounds were not detected in the secondary effluent by chemical analysis, but the samples had an estrogenic effect. The main ozonation formed oxidation by-products of the organic micropollutants but decreased the level of non-specific toxicity and other specific toxic modes of action, demonstrating that the mixture of oxidation by-products was less potent than the mixture of the parent compounds for the considered effects.

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1. Introduction

In the last decade, numerous studies around the world have demonstrated the presence of pharmaceutical compounds in domestic wastewaters. These compounds are removed to different degrees by the commonly used biological activated sludge processes. While some compounds (e.g. ibuprofen, paracetamol) are effectively removed, others (e.g. carbamazepine, diclofenac) are barely affected by the biological treatment (Onesios et al., 2009). As a result, some pharmaceutical compounds are released into the environment, contaminating surface waters. This situation is of concern as these compounds have been designed to affect biological systems and long term exposure effects on the biological elements in the environment are still largely unknown. Human health is also at risk when treated wastewater is discharged to water bodies that are used as drinking water sources or considered for indirect potable reuse. Therefore, advanced treatment of wastewaters has to be envisaged to reduce the load of these compounds in discharged effluents.

Several technologies have proven to be effective in removing pharmaceutical compounds from water: activated carbon adsorption (Nowotny et al., 2007; Snyder et al., 2007; Ternes et al., 2002; Westerhoff et al., 2005; Yu et al., 2008), ozonation and advanced oxidation processes (Esplugas et al., 2007; Huber et al., 2003; Huber et al., 2005; Kim et al., 2008; Nakada et al., 2007; Ternes et al., 2003; Zwiener and Frimmel, 2000) and membrane filtration (Kimura et al., 2004; Snyder et al., 2007; Yoon et al., 2007). Activated carbon adsorption and ozonation are considered to be economically feasible for tertiary treatment of wastewaters by Joss et al. (2008). Nevertheless, none of these processes can remove all the compounds of concern and to date, no extensive study has been carried out on the efficiency of the sequential combination of these two processes for the removal of micropollutants from secondary treated wastewater.

Chemical analysis of micropollutants in water has gone through major developments in recent years and today's analytical techniques can measure some compounds at concentrations as low as a few ng L^{-1}. Chemical analysis is powerful to measure the concentration of targeted compounds but does not cover the whole range of chemicals that might be present in the effluent of a wastewater treatment plant. Analytical techniques usually focus on pharmaceutically active parent compounds whereas a variable fraction is excreted from the human body as metabolites. Moreover, biological treatment of wastewater may form yet unidentified compounds through biodegradation reactions; e.g. 17β-estradiol is degraded to estrone (Ternes et al., 1999). Subsequent chemical treatments such as ozonation can also form by-products. Chemical analysis of a limited suite of compounds does not allow assessment of the potential biological adverse effects of the wastewater as the cumulative effect of the mixture of chemicals that may be present cannot be easily integrated. Bioassays have been utilised as complementary monitoring tools for the assessment of possible biological effects of chemicals that are present in a particular water sample (Escher et al., 2008b; Muller et al., 2007). These bioanalytical tools are designed to quantify non-specific toxicity or particular toxic modes of action (e.g. estrogenicity, genotoxicity, phytotoxicity) induced by a sample on a biological organism or a biological process. To date, bioassays have not been widely used in the evaluation of water treatment processes and have rarely been accompanied with chemical analysis (Macova et al., 2009).

The present study assessed the removal of selected micropollutants (pharmaceuticals and pesticides) and the decrease of biological activity along a full scale water reclamation plant using ozonation and activated carbon adsorption to treat a secondary effluent. This aimed at identifying the key steps in the treatment process as well as the key operating parameters. Results of chemical analysis and bioassays were compared to determine whether the use of both techniques was redundant or allowed a deeper understanding of the performances of individual treatment processes. The performance and relevance of the treatment train is further discussed in the context of indirect potable reuse.

2. Materials and methods

2.1. South Caboolture Water Reclamation Plant

The South Caboolture Water Reclamation Plant was designed to reduce riverine pollution from the 40,000 population equivalent wastewater treatment plant and to provide recycled water to industry and community consumers. Whilst the plant provides water for non-potable applications, it has been designed to meet drinking water standards. The treatment process detailed in Fig. 1 incorporates biological denitrification, pre-ozonation, coagulation/flocculation/dissolved air flotation-sand filtration (DAFF), main ozonation, biological activated carbon filtration and final ozonation for disinfection. The activated carbon was renewed in March 2008 after 9 years of service, its adsorption capacity was assumed not to be exhausted at the time sampling took place, 4 months later. van Leeuwen et al. (2003) published more details on the process and its performances.

2.2. Sample collection

Four sets of samples were collected over winter 2008 under dry weather conditions including three during week days and one during the weekend (11-07-08, 22-07-08, 27-07-08 and 06-08-08). Water temperature across the plant was 22 ± 2 °C and pH was 7.0 ± 0.5. Samples were collected at 7 sampling points along the treatment train, labelled S1 to S7 on Fig. 1, in order to evaluate the performance of individual treatment steps. As the flow rate in the reclamation plant is constant (8 megalitres per day), representative samples were collected as time proportional 24-h composites. For S1 and S7, refrigerated auto-samplers collected 200 mL every 15 minutes; for the remaining sampling locations, a continuous flow pump collected samples at a flow rate of 7 mL min^{-1}. At each point, samples were collected into a glass bottle pre-washed with MilliQ water and HPLC grade acetone. The samples were protected from light and refrigerated during collection. For micropollutant analysis, 1 L was transferred into solvent

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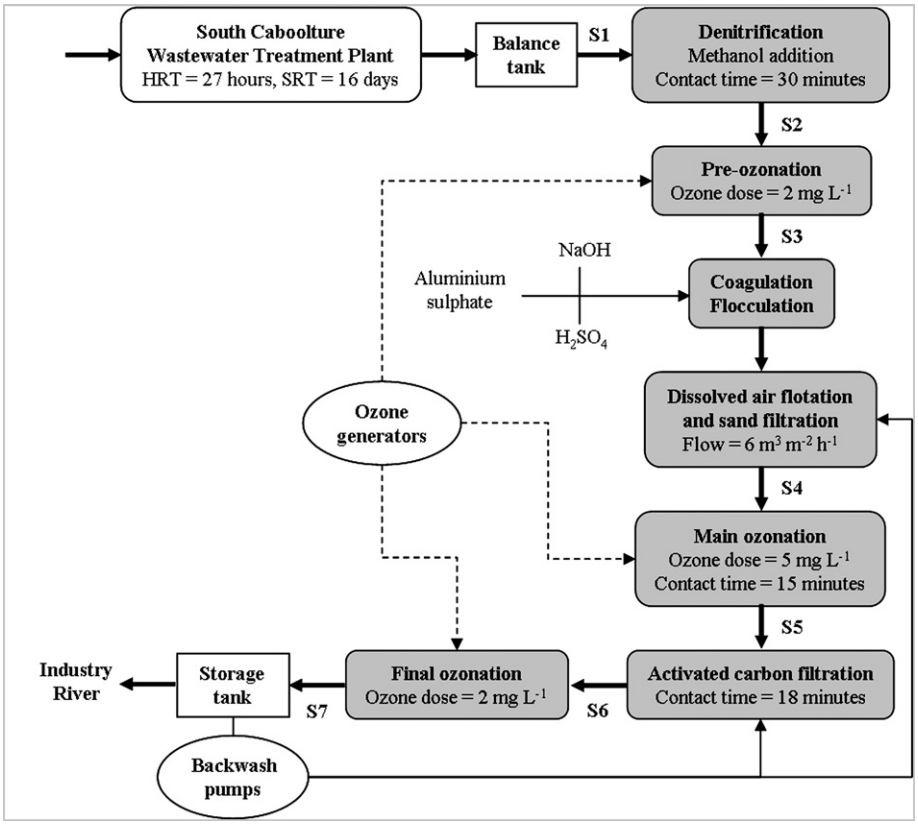

Fig. 1 – South Caboolture Water Reclamation Plant process (adapted from van Leeuwen et al. (2003)). S1 to S7: sampling locations. HRT: hydraulic retention time. SRT: sludge retention time.

washed amber glass bottles and preserved with sodium thiosulfate. For the bioassays, 2 L were transferred into solvent washed amber glass bottles and hydrochloric acid (36%) was added to a final concentration of 5 mM for preservation. For dissolved organic carbon (DOC) measurements, 100 mL were filtered through a 0.45 μm PTFE membrane and collected in plastic (HDPE) bottles rinsed with the sample beforehand. All samples were transported on ice and stored frozen or at 4 °C.

2.3. *Chemical analyses*

2.3.1. *Dissolved organic carbon*

The dissolved organic carbon (DOC) was measured as non-purgable organic carbon (NPOC) with an Analytik Jena multi N/C 3100 instrument at the Advanced Water Management Centre (AWMC). For each sample, 2—3 replicates were measured, giving a relative standard deviation of less than 3%.

2.3.2. *Micropollutants*

Micropollutant analysis was carried out by Queensland Health Forensic and Scientific Services (QHFSS, accredited by the National Association of Testing Authorities, Australia, and ISO 9001 certified). The method consisted of solid phase extraction (SPE), concentration and quantification by liquid chromatography coupled with tandem mass spectrometry (LC/MS—MS). This method, described in details in the supporting information SI 1, allowed the quantification of 85 compounds (listed in Table SI 3 in the supporting information SI 2) selected on the basis of quantity of usage of the particular compounds, their potential toxicity and their resistance to degradation. Their limit of quantification (LOQ) was 0.01 $\mu g L^{-1}$ in most cases. Concentrations were calculated using an internal calibration method. Results were not corrected for recovery efficiency of the extraction method. Recoveries of single compounds are assumed to be consistent along the treatment as the matrix is similar therefore; calculation of the removal efficiency is not affected. Indeed, Gros et al. (2009) determined the recoveries of 73 pharmaceutical compounds by SPE in raw wastewater and treated effluent and their results show that the recovery for a given compound generally vary by less than 20% from one matrix to the other despite the fact that these matrices are very different. The standard deviation of the method is 20% (Table SI 1 in the supporting information SI 1). When the reported outlet concentration was below the LOQ of the compound, removal efficiency was calculated as a minimum value using the LOQ as outlet concentration.

2.3.3. *Bioassays*

Six bioassays, discussed in details in Macova et al. (2009), were applied to the samples collected in the study. Baseline toxicity was determined with the non-specific *Vibrio fischeri* bioluminescence inhibition test. Five additional specific toxic modes of action were also evaluated: estrogenic activity (E-SCREEN assay), arylhydrocarbon receptor response (CAFLUX assay), neurotoxicity (acetylcholinesterase inhibition assay), phytotoxicity (PSII inhibition I-PAM assay) and genotoxicity (umuC

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assay). Water samples were extracted by SPE using Oasis HLB cartridges. Full dose response curves were determined for a serial dilution of the extract for each bioassay. Results were expressed as toxic equivalent concentrations (TEQ) except for the umuC assay. The TEQ represents the concentration of a given reference compound that would be required to produce the same effect as the mixture of the various compounds in the sample. When the outlet TEQ was below the LOQ of the bioassay, removal efficiency was calculated as a minimum value using the LOQ as outlet TEQ. In the umuC assay, the response is determined as an induction ration (IR), an $IR \geq 1.5$ is considered genotoxic. For genotoxic samples, $EC_{IR1.5}$ corresponds to how many times the sample must be concentrated or diluted to elicit an IR of 1.5. Results are expressed as $1/EC_{IR1.5}$ therefore a higher number represents a higher genotoxic effect.

3. Results and discussion

3.1. *Micropollutants*

In the reclamation plant's influent, 54 of the 85 targeted compounds had a median concentration above their LOQ confirming that conventional activated sludge treatment does not completely remove these micropollutants from wastewater (Fig. 2). The concentrations ranged from 0.01 to 2.10 $\mu g L^{-1}$ with the exception of gabapentin, which was consistently found at higher concentrations ranging from 5.60–6.50 $\mu g L^{-1}$ (data provided in the supporting information in Table SI 3). The factor between the minimum and the maximum concentrations measured for each individual compound was generally close to or lower than 2, with a maximum of 3.6 observed for iopromide. No clear pattern could be distinguished between the different sampling days. The increase or decrease of single compound concentrations from one day to another appeared to be random, even when comparing the sample collected during the weekend to samples collected during week days. Twenty-five compounds had an influent median concentration above 0.10 $\mu g L^{-1}$, their fate is further detailed hereafter. The removal efficiency of these compounds was determined in each treatment step except when the concentration before treatment was lower than ten times the LOQ and below LOQ after treatment. This criterion was used to allow the determination of removals up to 90% in any case. The DOC was measured for two sets of samples (22-07-08 and 06-08-08), and varied from 14.2 to 19.7 $mg L^{-1}$ in the influent water. The following paragraphs discuss the performance of each individual stage of the treatment by comparing concentrations of the selected compounds in the inlet and outlet water for the considered process.

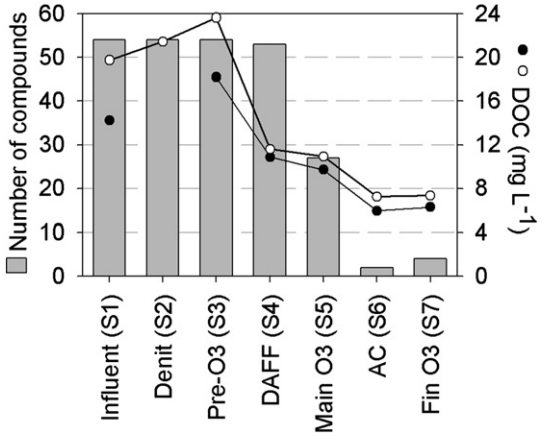

Fig. 2 – Number of compounds quantified and dissolved organic carbon (DOC) after indicated stage along the treatment train. Bars represent the number of compounds (left y-axis) with a median concentration above the LOQ (4 samples). Dots represent DOC on two different sampling days (right y-axis). Denit: denitrification; Pre-O3: pre-ozonation; DAFF: dissolved air flotation-sand filtration; Main O3: main ozonation; AC: activated carbon; Fin O3: final ozonation.

3.1.1. *Denitrification*

The number of compounds above LOQ was unchanged after denitrification and the concentrations of the 25 selected compounds decreased by less than 20% (with the exception of atenolol, 38%). To our knowledge, the removal of micropollutants by denitrifying bacteria has not been specifically studied to date. In the present case, methanol is added to the water prior to the denitrification reactor and the bacteria use it as the primary electron donor which may prevent or reduce the degradation of micropollutants. The observed increase in DOC after the denitrification was probably due to residual methanol.

3.1.2. *Pre-ozonation*

The number of compounds quantified was stable through the pre-ozonation process (Fig. 2) and the concentration of the 25 selected compounds decreased by less than 30%. The ozone dose applied in the pre-ozonation step is approximately 2 $mg L^{-1}$ and the DOC was approximately 20 $mg L^{-1}$ corresponding to an ozone dose of 0.1 $mg_{O3}\ mg_{DOC}^{-1}$. In such conditions, ozone is rapidly consumed by the organic matter (Buffle et al., 2006a,b) therefore micropollutants oxidation is limited. Ozonation has been proved to be very effective for oxidising various micropollutants in secondary treated wastewaters (Hollender et al., 2009; Huber et al., 2005; Snyder et al., 2006; Ternes et al., 2003; Wert et al., 2009) but with higher ozone doses of at least 0.25 to 0.50 $mg_{O3}\ mg_{DOC}^{-1}$. Snyder et al. (2006) also investigated a full scale wastewater treatment plant using pre-ozonation with a low ozone dose (not specified) prior to biological activated carbon filtration, and similarly, observed removal of micropollutants inferior to 30%.

3.1.3. *Coagulation/flocculation/DAFF*

In the coagulation/flocculation/DAFF process, the negatively charged colloids are neutralised by the addition of a chemical agent (aluminium sulphate) which causes them to aggregate in floccs (flocculation) which are subsequently removed by dissolved air flotation and sand filtration. The main purpose of this stage is to decrease the DOC, which was achieved effectively as demonstrated by the 40–50% DOC reduction (Fig. 2). This reduction of the DOC is important to achieve maximal

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performance of the main ozonation process as discussed above. After the DAFF, 53 compounds were quantified and the concentrations of the 25 selected compounds decreased by less than 20% except for atenolol (42%), caffeine (29%), gabapentin (44%), gemfibrozil (32%) and roxithromycin (37%). It is also interesting to note that paracetamol concentration increased by 44% in the process, which could not be explained. No literature reporting investigations of the removal of micropollutants from treated wastewaters by coagulation-flocculation could be identified. However, several studies on lab-scale and full scale drinking water treatment systems have reported removals lower than 50% for several pharmaceuticals and pesticides by such solids removal processes (Adams et al., 2002; Ternes et al., 2002; Thuy et al., 2008; Vieno et al., 2006; Westerhoff et al., 2005). Suarez et al. (2009) also showed similar limited removal in hospital wastewaters. Nevertheless, Suarez et al. (2009) and Westerhoff et al. (2005), showed that removals of up to 80% can be achieved for highly hydrophobic compounds (i.e $\log K_{ow} > 6$) suggesting that the micropollutants removal during coagulation-flocculation occurs via hydrophobic interactions with neutral particles. Most of the pharmaceuticals and pesticides measured in this study are hydrophilic or of moderate hydrophobicity with $\log K_{ow} < 4$ (Table SI 3 in supporting information SI 2); therefore little removal can be expected in the coagulation/flocculation/DAFF stage. Among the exceptions observed, the removal of gemfibrozil can be explained by its more hydrophobic nature ($\log K_{ow} = 4.77$, EPI SUITE 4.0) but other compounds are very hydrophilic and no explanation for their removal could be advanced.

3.1.4. *Main ozonation*

The main ozonation stage (approximately 0.5 mg_{O3} mg^{-1}_{DOC}) decreased the concentration of 26 compounds below LOQ (Fig. 2). Among the 25 selected compounds, 9 showed a reduction of more than 90% and 13 others were reduced by more than 70% while iopromide and gabapentin were reduced by 55% (Fig. 3). Removal of naproxen was not determined because its concentration was lower than ten times its LOQ before treatment and below LOQ after. The higher removal efficiency obtained by the main ozonation stage compared with the pre-ozonation is due to the higher ozone dosage relative to the DOC content. These results are in agreement with previous findings (Buffle et al., 2006b; Hollender et al., 2009; Huber et al., 2005; Snyder et al., 2006; Ternes et al., 2003; Wert et al., 2009). The reaction of organic compounds with molecular ozone is selective and only certain groups of compounds react rapidly, e.g. aliphatic molecules with double bonds, deprotonated amines and aromatics with an activating group. Most of the pharmaceuticals and pesticides possess one of these moieties and would be expected to react with molecular ozone with high second order rate constants (k_{O3}). The results show that the removal of micropollutants increased with increasing value of k_{O3} but even compounds weakly reactive with ozone (i.e. iopromide and MCPA; Table 1) were oxidised. Oxidation in ozonation processes can also occur via the attack of hydroxyl radicals formed by ozone decomposition in water. Hydroxyl radicals are highly reactive with almost any organic molecule; the second order rate constants of oxidation by hydroxyl radicals are typically in the 10^9 M^{-1} s^{-1} range (Table 1). Therefore, the observed removals of iopromide and MCPA suggest a substantial exposure to hydroxyl radicals. Indeed, Buffle et al. (2006a,b) showed that the ozonation of wastewaters leads to high exposure to hydroxyl radicals; in the first instants of the reaction, the hydroxyl radical production is equivalent to, or greater than what can be obtained with advanced oxidation processes. To our knowledge, gabapentin oxidation by ozone has not been reported in the literature and no reaction rate data could be found. Gabapentin possesses both carboxylic and amine

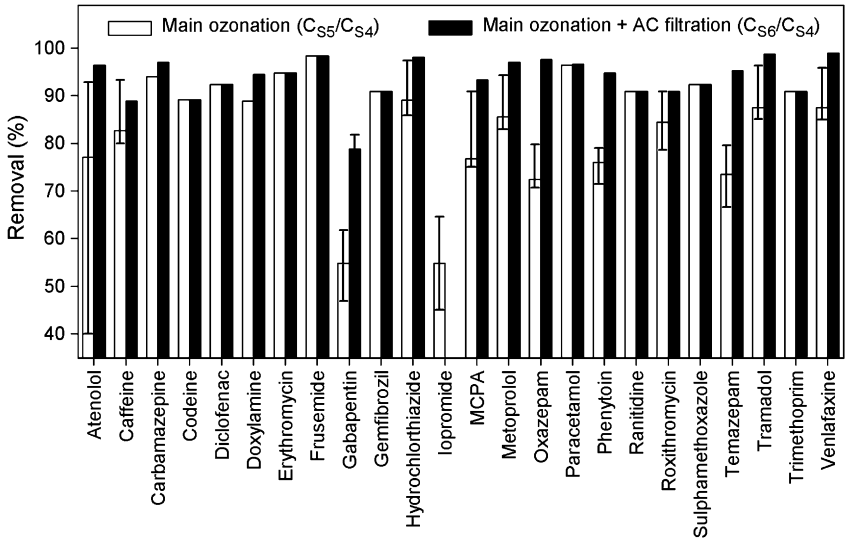

Fig. 3 – Median removal of selected compounds (median of influent concentration > 0.10 μg L^{-1}) by the main ozonation stage and the combination of the main ozonation and the activated carbon (AC) filtration stages. Error bars represent minimum and maximum removal, no error bar means that the compound was below LOQ after treatment; therefore removal was calculated as a minimum using the LOQ. C_{S4}: concentration before main ozonation; C_{S5}: concentration after main ozonation; C_{S6}: concentration after activated carbon filtration.

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Table 1 – Removal of selected pharmaceuticals in the main ozonation stage and their first order reaction rate constants with ozone (k_{O3}) and hydroxyl radicals ($k_{HO\cdot}$). The temperature (T) and pH given are the experimental conditions used for the determination of the reaction rates.

Compound	Removal	k_{O3} ($M^{-1}\,s^{-1}$)	pH	T (°C)	$k_{HO\cdot}$ ($109.M^{-1}\,s^{-1}$)	pH	T (°C)	Ref.
Iopromide	55%	<0.08	7	20	3.3 ± 0.6	7	25	a
MCPA	77%	47.7	2	20	6.6	9	20	b
Atenolol	77%	$(1.7 \pm 0.4) \times 10^3$	7	20–22	8.0 ± 0.5	7	20–22	c
					7.05 ± 0.27	7	room	d
Roxithromycin	84%	7×10^4	7	20				a
		6.3×10^4	7	20	5.4 ± 0.3	7	25	e
Metoprolol	86%	$(2.0 \pm 0.6) \times 10^3$	7	20–22	7.3 ± 0.2	7	20–22	c
					8.39 ± 0.06	7	room	d
Ranitidine	>91%	3.0×10^4–1.0×10^6	2	20				f
Trimethoprim	>93%	2.7×10^5	7	20	6.5 ± 0.2	7	25	e
Sulphamethoxazole	>93%	~2.5×10^6	7	20	5.5 ± 0.7			a
		5.5×10^5	7	20		7	25	e
Diclofenac	>94%	~10^6	7	20	7.5 ± 1.5			a
		$(1.84 \pm 0.15) \times 10^4$	6	25		7	25	g
Paracetamol	>96%	4.11×10^6 [k]	7	25	2.2 ± 0.017	5.5	25	h
Carbamazepine	>98%	~3×10^5	7	20	8.8 ± 1.2	7	25	a
		$(7.81 \pm 1.31) \times 10^4$	25					i
					2.05 ± 0.14	5	room	j

a Huber et al. (2003).
b Benitez et al. (2004).
c Benner et al. (2008).
d Song et al. (2008).
e Dodd et al. (2006).
f Rivas et al. (2009).
g Vogna et al. (2004b).
h Andreozzi et al. (2003).
i Andreozzi et al. (2002).
j Vogna et al. (2004a).
k Calculated from Andreozzi et al (2003).

groups, their pK_a are 3.89 and 9.56 respectively (Zour et al., 1992). Therefore gabapentin is present as a zwitterion under usual wastewater pH conditions. Protonated amines and deprotonated carboxylic acids are typically not highly reactive with ozone (Hoigné and Bader, 1983) and gabapentin oxidation can be expected to be controlled by hydroxyl radical reactions. As a primary amine, gabapentin has an expected second order rate constant for the reaction with ozone of about 50 $M^{-1}\,s^{-1}$ which is significantly higher than for iopromide. However, the similar behaviour of the two substances shows that their decrease is controlled by hydroxyl radical reactions. The DOC decreased by less than 10% in the process, showing that ozonation does not lead to substantial mineralization of dissolved organic matter.

3.1.5. *Activated carbon filtration*

Activated carbon filtration further removed the micropollutants and only 2 compounds were quantified above their LOQ downstream: roxithromycin (0.01 $\mu g\,L^{-1}$) and gabapentin (0.70 $\mu g\,L^{-1}$). The removal efficiency of the compounds having a median concentration of at least ten times their LOQ prior to activated carbon filtration was calculated: oxazepam, tramadol and venlafaxine were removed by more than 90% and gabapentin was removed by 53%. Adsorption onto activated carbon also removed 20–30% of the DOC showing a less effective adsorption of organic matter than of micropollutants. Previous studies (Ormad et al., 2008; Snyder et al., 2007; Ternes et al., 2002; Westerhoff et al., 2005) demonstrated that powdered and granular activated carbon can efficiently remove micropollutants from natural water sources used for drinking water. To date, the removal of micropollutants from wastewaters by activated carbon has not been extensively studied; Snyder et al. (2007) found that a water reuse facility using granular activated carbon without regular replacement/regeneration provided little removal. In the present work, the activated carbon had been replaced about 4 months before the collection of the samples so it is assumed that its adsorption capacity was not yet exhausted. Adsorption onto activated carbon is driven mainly by hydrophobic interactions and Westerhoff et al. (2005) observed that the trend in the removal efficiency of micropollutants by activated carbon can be predicted from logK_{ow} values. Gabapentin is zwitterionic at neutral pH as shown above and much more hydrophilic (logK_{ow} = -1.37) compared to oxazepam, tramadol and venlafaxine (logK_{ow} = 2.32, 3.01 and 3.28 respectively). This explains the difference observed in the removal efficiencies of these compounds. It can be expected that, with time, the adsorption capacity of the activated carbon filter will decrease and eventually be exhausted while biological activity will develop in the filter and contribute to biodegradation of the by-products formed by ozonation (Simpson, 2008).

3.1.6. *Final ozonation*

In the final effluent, after final ozonation, 4 compounds had a median concentration above the LOQ: gabapentin (0.45 $\mu g\,L^{-1}$), roxithromycin (0.01 $\mu g\,L^{-1}$), DEET (0.03 $\mu g\,L^{-1}$) and caffeine

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(0.02 $\mu g\,L^{-1}$). The median concentrations of caffeine and DEET are very close to the LOQ and are below the LOQ prior to the final ozonation stage; therefore one can assume that their quantification is due to analytical variations rather than an actual concentration variation. It is difficult to evaluate the efficiency of the final ozonation in the removal of micropollutants because they all had concentrations equal or below the LOQ before the treatment except gabapentin. The median removal of gabapentin in the final ozonation was only 20%, which is low compared to the main ozonation, and is due to the lower ozone dose employed (approximately 0.3 $mg_{O3}\ mg^{-1}_{DOC}$). The DOC was not removed in this treatment step.

3.1.7. *Overall treatment*

The full treatment decreased the concentration of 50 of the 54 compounds quantified in the influent water to levels below LOQ (Fig. 2). Concomitantly, DOC was also reduced by 55–60% in the effluent compared to the influent water. Overall, among the 25 selected compounds, 11 were removed by more than 95% and 11 by more than 89%. The median removal of gabapentin was 86% and the removals of naproxen and iopromide were not calculated because their concentration was lower than 10 times their LOQ in the influent and below their LOQ in the effluent. The 4 remaining compounds were gabapentin (0.45 $\mu g\,L^{-1}$), roxithromycin (0.01 $\mu g\,L^{-1}$), DEET (0.03 $\mu g\,L^{-1}$) and caffeine (0.02 $\mu g\,L^{-1}$).

The first 3 stages of the treatment train (i.e. denitrification, pre-ozonation and coagulation/flocculation/DAFF) did not decrease the number of micropollutants quantified in the water (Fig. 2). After these 3 stages, the concentrations of the 25 compounds that had an influent median concentration of at least 0.10 $\mu g\,L^{-1}$ were generally still greater than 50% compared to the influent concentration (Fig. 4). The main ozonation and the activated carbon adsorption played a key role in the treatment bringing the concentration of 26 and 25 compounds below LOQ respectively (Fig. 2). The main ozonation generally decreased the micropollutants to less than 20% of their influent concentration and activated carbon filtration further removed the compounds to levels below LOQ except for gabapentin and roxythromycin (Fig. 4). The combined effects of the main ozonation and the activated carbon filtration processes decreased the concentration of 10 of the 25 selected micropollutants by more than 95% and by more than 89% for 12 of the 15 remaining compounds compared to their concentration prior to the main ozonation (Fig. 3). Gabapentin concentration was reduced by 79%. These results show that ozonation followed by activated carbon filtration is a very effective combination of processes to remove micropollutants from secondary treated wastewater. The key steps in the removal of the DOC were the DAFF and the activated carbon adsorption. Although the DAFF reduced the concentration of micropollutants by less than 30%, it also played a key role indirectly by reducing the DOC which enhanced the performances of the main ozonation.

3.2. *Bioanalytical results*

The influent biological activity was higher than the blank (MilliQ water) in all the bioassays (Table 2). The effect of the treatment train on each bioassay is discussed individually hereafter.

3.2.1. *Baseline toxicity*

The *Vibrio fischeri* bioluminescence inhibition test is a non-specific bacterial toxicity test widely recognised in the field of ecotoxicology as the standard assay for acute cytotoxicity. The

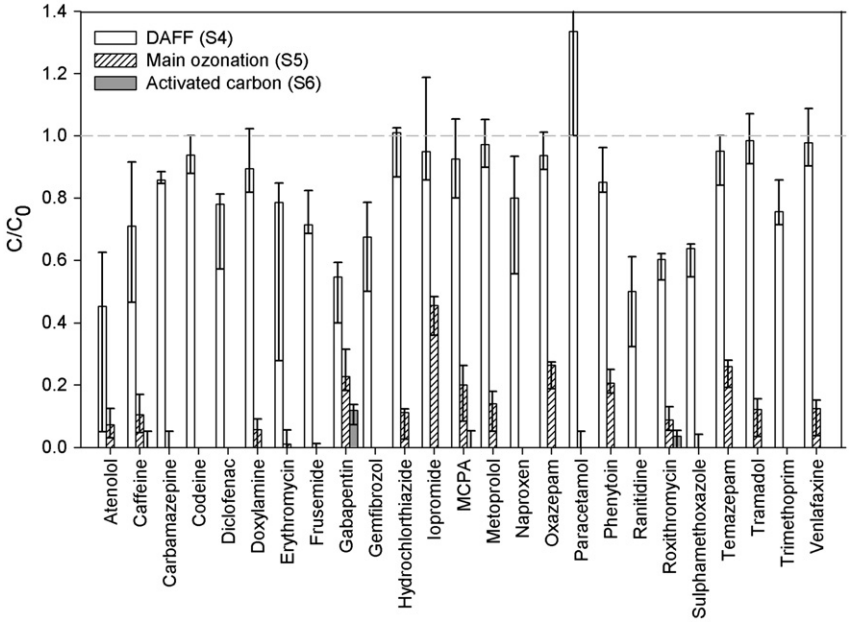

Fig. 4 – Median relative concentrations of selected compounds (median of influent concentration > 0.10 g L^{-1}) after the dissolved air flotation-sand filtration (DAFF, S4), the main ozonation (S5) and the activated carbon (S6) stages (error bars represent maximum and minimum values). C is the concentration after the specified treatment step and the reference concentration, C_0, is the concentration in the influent water of the reclamation plant.

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Table 2 – Maximum, median and minimum biological activity in the influent and effluent of the reclamation plant and overall maximum, median and minimum decrease observed.

Bioassay	Result expression	Blank	Influent			Effluent			Decrease (%)		
			Max	Med	Min	Max	Med	Min	Max	Med	Min
Baseline toxicity	Baseline toxicity EqC[a] (TEQ, mg L^{-1})	0.21	2.9	2.1	2.0	0.72	0.52	0.31	84	78	67
Estrogenicity	Estradiol EqC (EEQ, ng L^{-1})	<0.02	7.6	5.8	5.7		<0.06				>99
AhR response	TCDD EqC (TCDDEQ, ng L^{-1})	0.08	0.98	0.82	0.59	0.36	0.31	0.26	69	62	46
Genotoxicity	$1/EC_{IR1.5}$	<0.01	0.32	0.19	0.13	0.04	<0.01			>93	82
Neurotoxicity	Parathion EqC (PTEQ, μg L^{-1})	<0.3	3.9	3.1	2.8	1.2	<0.3			>90	57
Phytotoxicity	Diuron EqC (DEQ, μg L^{-1})	<0.01	0.22	0.10	0.05	0.05	0.03	<0.01	>91	75	50

a EqC = equivalent concentration.

assay reflects the general ''energy status'' of the bacteria and can indicate the toxic potency of a broad spectrum of compounds with different modes of action. Denitrification and pre-ozonation did have a slight stimulatory effect on the baseline toxicity whereas the following treatment steps were able to substantially decrease baseline toxicity (Fig. 5). The TEQ was reduced to 62, 37 and 21% of the influent water level after the coagulation/flocculation/DAFF, the main ozonation and the activated carbon filtration respectively. As is discussed in more details in Macova et al. (2009), an almost linear correlation exists between DOC level and TEQ. The slight increase in TEQ after denitrification cannot be related to the residual methanol added before this process, which is still partially present after denitrification as is discussed above, but is likely to be related to some non-volatile organic chemicals. The 52% decrease of TEQ in the DAFF stage is accompanied by a 40–50% reduction in DOC, whereas micropollutants concentrations were generally reduced by less than 30% in the meantime. Although the SPE that is performed prior to toxicity testing should be able to remove a substantial fraction of the DOC and will remove all of the residual methanol, some DOC, most likely smaller breakdown products that have similar physicochemical properties and similar molecular weight, may still be present. Consequently, the baseline toxicity is, at least partially, induced by compounds having a fate differing from the fate of measured micropollutants in the treatment process and that chemical analysis alone cannot effectively assess the effect of the treatment processes on water quality.

The main ozonation reduced the TEQ by 31% whereas the concentration decay observed for most of the micropollutants was higher than 70%. The DOC was not affected. It is known that some organic compounds are poorly reactive with ozone and the results of the micropollutant analysis showed that some compounds were only partially degraded in the main ozonation step (i.e iopromide and gabapentin). Moreover, ozonation does not typically lead to complete mineralization but to the formation of by-products. The oxidation products of ozonation are in general more polar and more hydrophilic molecules than the parent compounds but the modification is not drastic. Therefore the oxidation products of ozonation will still have a considerable effect in a non-specific assay like the bioluminescence inhibition test with *Vibrio fischeri*, where the toxicity is generally directly related to the hydrophobicity of the mixture components (Escher et al., 2008a).

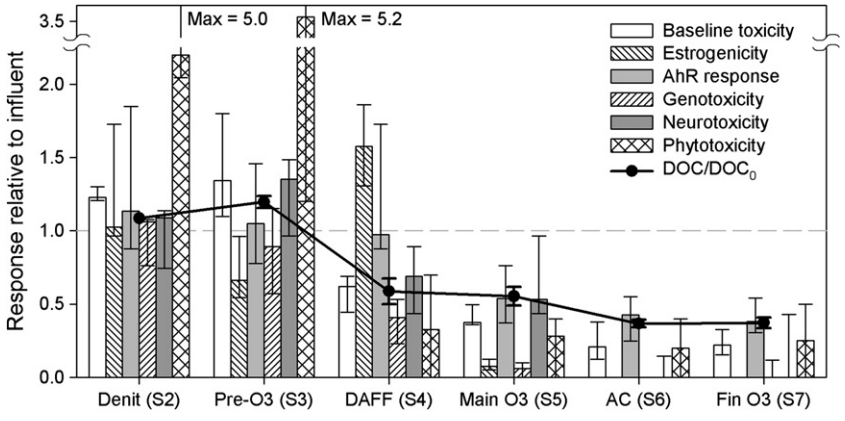

Fig. 5 – Relative response of the bioassays and relative dissolved organic carbon (DOC) after indicated stage along the treatment train compared to the influent (DOC_0 = influent DOC). Bars are the median of 4 values for bioassays and error bar represent maximum and minimum values. Dots are the average of 2 values for DOC and error bars represent maximum and minimum values. Denit: denitrification; Pre-O3: pre-ozonation; DAFF: dissolved air flotation-sand filtration; Main O3: main ozonation; AC: activated carbon; Fin O3: final ozonation. Baseline toxicity: *Vibrio fischeri* bioluminescence inhibition test; estrogenicity: E-SCREEN assay; arylhydrocarbon (AhR) receptor response: CAFLUX assay; genotoxicity: umuC assay; neurotoxicity: acetylcholinesterase inhibition assay; phytotoxicity: PSII inhibition I-PAM assay.

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Activated carbon filtration reduced the baseline toxicity by 50% and the DOC by 30 to 35%. Activated carbon effectively adsorbs the more hydrophobic compounds, which is again consistent with the general trend discussed above that the more hydrophobic compounds have a higher toxic activity than the more hydrophilic ones. Based on this fact, identification of the compounds exhibiting a high toxic activity could start with the identification of the more hydrophobic compounds.

The final ozonation did not further reduce the baseline toxicity compared to after activated carbon filtration. The effluent TEQ was approximately 80% lower than the influent TEQ (Fig. 5) and only 2.5 times higher than the blank (Table 2). This indicates that the residual toxicity is of no concern, unless the residual organic chemicals and organic matter inducing this effect were of very specific potency. This latter question was tested with a series of specific endpoints that respond to environmentally relevant modes of toxic action.

3.2.2. *Estrogenic activity*

The E-SCREEN assay specifically responds to natural hormones and other compounds that can mimic the activity of the female sex hormone estradiol. The estrogenic activity of the samples is expressed as an estradiol equivalent concentration (EEQ). The median influent EEQ was 5.8 ng L^{-1}; higher than levels previously reported in South East Queensland. Most of the effluents from 12 activated sludge wastewater treatment plants tested by Leusch et al. (2006) had EEQs below 4 ng L^{-1} and sometimes below 1 ng L^{-1}. Hormones and endocrine disrupting compounds (EDCs) were not investigated in this study but an earlier sampling campaign of the secondary treated wastewater showed that the concentrations of measured estrogenic compounds (17β-estradiol, 17α-ethynylestradiol, estrone, estriol, bisphenol A, nonylphenol) were all below the LOQ of 1 ng L^{-1} (Table SI 4 in supporting information SI 3). This demonstrates the relevance of using bioassays as complementary tools to chemical analysis for the assessment of water quality and process performances.

Denitrification did not affect the estrogenicity (Fig. 5). Preozonation with an ozone dose of approximately 0.10 mg_{O3} mg^{-1}_{DOC} reduced the EEQ by 34% compared to the influent. This is higher than the removal previously observed by Snyder et al. (2006) who measured the EEQ reduction induced by various ozone doses in treated wastewater with a DOC of 6.38 mg L^{-1}. They found that an ozone dose of 2.1 mg L^{-1} (0.33 mg_{O3} mg^{-1}_{DOC}) only removed 18% of the EEQ but, with ozone doses of 3.6 mg L^{-1} (0.56 mg_{O3} mg^{-1}_{DOC}) and above, 90% or more removal could be achieved. In a recent study on a full scale ozonation in a Swiss sewage treatment plant (STP), the dose-dependency of removal of micropollutants yielded similar results (Escher et al., 2009). While most endpoints showed a clear dose-dependency of reduction of effects, the reduction of estrogenicity was already large at low ozone doses and depended more on the EEQ than on the ozone dose. When estrogenicity was already below a certain level, which was very close to the detection limit, the quantification of further reduction became difficult and prone to large uncertainty. For the remaining samples, ozone doses of 1.6–5.3 mg L^{-1} in the presence of 4.2–6.0 mg L^{-1} DOC lead to more than 90% reduction of estrogenicity. This is consistent with laboratory experiments that demonstrated that almost all first-generation transformation products of estrogenic chemicals had severely decreased estrogenic potency (Lee et al., 2008). Thus ozonation can be considered as a fairly selective oxidation, where even low doses selectively target one of the most environmentally relevant modes of toxic action, namely estrogenicity.

After the coagulation/flocculation/DAFF stage the EEQ increased drastically by a median factor of 3.3 compared to the level prior to treatment. At this treatment step, the concentration of DOC is greatly reduced (by 40–50%), and there is a likelihood that the estrogenic chemicals that were bound to DOC were released during this treatment step. We have previously observed with another estrogenicity assay that DOC appears to reduce the bioavailability of estrogens (B. Escher, unpublished results). Estrogenic chemicals are typically relatively hydrophobic and bind well to DOC (Neale et al., 2008). In general DOC is not bioavailable in bioassays (the discussion on the small breakdown products above is an exception of this general paradigm) and micropollutants sorbed to DOC would not be bioavailable either. A large fraction of the matrix and also the DOC is supposed to be removed by SPE but, given the color of the extracts, it is possible that a substantial fraction of larger DOC is co-extracted. In addition, for the E-SCREEN test, it was demonstrated that the presence of serum proteins modulates the free and bioavailable concentration of estrogenic chemicals (Heringa et al., 2004). This effect was also hydrophobicity dependent and was much more pronounced for the more hydrophobic octylphenol than for the less hydrophobic estradiol. Protein binding is generally less important than binding to DOC or lipids, therefore while the effect on bioavailability was not very large for estradiol in the study of Heringa et al. (2004); it might well be relevant under the conditions of the present study. This hypothesis needs to be evaluated in the future by exploring the correlation between size distribution of naturally occurring DOC and effect on bioavailability, estrogenicity and toxicity.

The main ozonation reduced the EEQ by a median value of 92 and 95% compared to the level of the reclamation plant's influent and to the level before treatment respectively whereas DOC was not affected. It can be concluded that the mixture of by-products formed by the oxidation of the estrogenic compounds by ozone and hydroxyl radicals have a much lower estrogenic activity than the mixture of parent compounds, which is consistent with expectations as discussed above and in Lee et al. (2008).

Activated carbon filtration was able to efficiently adsorb residual estrogenic compounds and further reduced the EEQ by another 95% to levels below the detection limit of 0.02 ng L^{-1} and the final effluent concentration was below the quantification limit of 0.06 ng L^{-1}. The overall treatment efficiency for the removal of estrogenic activity was greater than 99%. This is in good agreement with observations on a full scale ozonation in a Swiss STP (Escher et al., 2009). As discussed above the analytically determined concentrations of (xeno)estrogens were below the quantification limit, therefore for this endpoint the very sensitive bioassay poses a great advantage despite the observed limitations due to matrix effects.

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3.2.3. *Ah-receptor response*

The CAFLUX assay targets dioxins and dioxin-like compounds such as polychlorinated biphenyls (PCBs) but can also respond to other chemicals such as polycyclic aromatic hydrocarbons (PAHs) (Macova et al., 2009). The results of the test are expressed as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalent concentration (TCDDEQ). The median TCDDEQ of the influent water was 0.82 ng L^{-1} and there was no significant variation along the first three steps of the treatment process; i.e denitrification, pre-ozonation and coagulation/flocculation/DAFF (Fig. 5). The main ozonation removed about 50% of the TCDDEQ but subsequent activated carbon filtration and final ozonation did not show further important removal and the median TCDDEQ of the final effluent was approximately 3.9 times higher than the blank (Table 2). Two sets of samples were submitted to a sulphuric acid silica gel clean up procedure that aims at removing organic chemicals except those that are not oxidised such as polychlorinated dibenzodioxins, -furans and PCBs. The samples were then tested again with the CAFLUX assay to evaluate the contribution of these very persistent chemicals (i.e dioxins, furans and dioxin-like PCBs). Results showed that after clean up the TCDDEQ was not significantly different from the blank (values ranged from 0.09 to 0.11 ng L^{-1}). This shows that the effect induced by the samples without sulphuric acid silica gel clean up is not due to the presence of dioxins, furans or dioxin-like PCBs but was caused by other chemicals. Since none of these groups of chemicals were quantified by chemical analysis in this study, no comparison between chemical and biological analysis is possible.

3.2.4. *Genotoxicity*

The umuC assay responds specifically to genotoxic compounds that cause DNA damages. To detect genotoxic effects caused by metabolites, the test is also performed in presence of a rat liver extract that can transform indirect genotoxicants to metabolites that are DNA damaging compounds. The median influent $1/EC_{IR1.5}$ were 0.19 and 0.060 in the absence and presence of the rat liver extract respectively, showing that the sample was less genotoxic after metabolisation. This is what one would commonly expect, an exception would be PAHs that are activated by metabolism. Denitrification and pre-ozonation did not have a substantial influence on genotoxicity (Fig. 5). The coagulation/flocculation/DAFF stage decreased $1/EC_{IR1.5}$ by 59% compared to the influent. The main ozonation drastically reduced the genotoxicity, $1/EC_{IR1.5}$ was reduced by 80 and 93% compared to the DAFF effluent and to the influent of the plant respectively. After activated carbon filtration as well as in the final effluent, $1/EC_{IR1.5}$ was below the LOQ of the bioassay (Table 2). In every case, the genotoxicity of the metabolised sample was lower than the non-metabolised sample, indicating that the type of chemicals inducing the genotoxic effect did not change over the treatment.

3.2.5. *Neurotoxicity*

Neurotoxicity is measured by the inhibition of the enzyme acetylcholinesterase (AChE). Organophosphate and carbamate pesticides specifically bind to this enzyme and the results are expressed as parathion equivalent concentration (PTEQ). The median PTEQ in the secondary treated wastewater was 3.1 μg L^{-1}; denitrification and pre-ozonation did not reduce the PTEQ whereas DAFF decreased it by 31% compared to influent (Fig. 5). Unlike the other bioassays, the effect of the main ozonation on PTEQ was not significant but activated carbon filtration reduced it drastically to level below the quantification limit of the bioassay (0.30 μg L^{-1}) which represents more than an 80% and 90% decrease compared to the main ozonation effluent and the plant influent water respectively. This observation is consistent with theoretical expectation, as it is known that compounds like diazinon and chlorpyrifos, which often constitute a large fraction of the acetylcholinesterase inhibitors, are not well oxidised by ozone. In contrast, these compounds are fairly hydrophobic ($\log K_{ow} = 3.96$ and 4.66 respectively), therefore sorption to activated carbon can be expected. A similar removal pattern has been observed for acetylcholinesterase inhibitors in the above-mentioned Swiss STP: none of the single removal steps (biological treatment, ozonation, sand filtration) had a high removal efficiency but all steps taken together produced a satisfactory overall removal (Escher et al., 2009).

3.2.6. *Phytotoxicity*

The I-PAM assay is sensitive to herbicides that directly inhibit photosynthesis; the results are reported as a diuron equivalent concentration (DEQ). The DEQ of the influent water ranged from 0.05 to 0.22 μg L^{-1} with a median value of 0.10 μg L^{-1} (Table 2). Diuron concentrations were measured by chemical analysis; it was reported in every sample of the influent water from 0.02 to 0.04 μg L^{-1}, suggesting that its contribution to the effect observed was limited. Among the other herbicides quantified, only simazine is also a photosystem II inhibitor with a relative potency of 0.15 (Muller et al., 2008). Simazine concentrations in the influent ranged from 0.05 to 0.19 μg L^{-1}. These two compounds considered together accounted for 17–93% of the measured DEQ demonstrating the interest of this bioassay to take into account non-measured compounds. The DEQ increased by factors of 2.2 and 3.5 after denitrification and pre-ozonation respectively but variation from one day to another was large therefore it is difficult to draw a conclusion (Fig. 5). This increase was accompanied with a slight increase in baseline toxicity and could therefore be caused by baseline toxicants interfering with the measurement of the photosynthesis yield (Macova et al., 2009). The coagulation/filtration/DAFF stage reduced DEQ by 67% and 88% compared to the plant's influent water and to the pre-ozonated water respectively. After the main ozonation the DEQ was not further significantly reduced; Diuron's concentrations were equal to or below the LOQ of 0.01 μg L^{-1} and simazine was removed by approximately 50%, their contribution accounting for 16–38% of the observed DEQ. Subsequent activated carbon filtration and final ozonation did not further affect the DEQ but diuron and simazine were removed below their LOQ. The overall treatment achieved 75% median decrease of DEQ, the effluent median DEQ was 0.03 μg L^{-1} (Table 2).

3.2.7. *Overall treatment*

The biological activity of the effluent was lower compared to the influent and close or equal to the blank level showing that the treatment process could effectively decrease the biological adverse effects observed with the bioassays; from 62% for the AhR response to more than 99% for estrogenicity (Table 2). The

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key treatment steps responsible for the decrease of biological activity are the DAFF stage, the main ozonation and the activated carbon adsorption (Fig. 5).

3.3. *Indirect potable reuse considerations*

Micropollutants reported concentrations were compared to the guidelines values for indirect potable reuse given in the Australian Guidelines for Water Recycling: Augmentation of Drinking Water Supplies (Table SI 3 in supporting information SI 2). The reported concentrations of the measured compounds were found to be below the guideline values in the influent water of the reclamation plant before any treatment. After removal by the advanced treatment process, concentrations were several orders of magnitude below the guideline values.

For information purpose, median equivalent concentrations obtained with the bioassays were compared to the corresponding reference compound's guideline value when available. Note however, that the effect caused by a mixture cannot be compared directly to a guideline value of a single compound. Moreover, the bioassays used here are acute tests and no conclusions can be drawn about chronic effects. Nevertheless such a comparison gives an impression on the expected hazard of the mixture but must be communicated with caution to a lay audience. For estrogenicity, neurotoxicity and phytotoxicity the reference compounds were estradiol, parathion and diuron and the guidelines values were 175 $ng\,L^{-1}$, 10 $\mu g\,L^{-1}$ and 30 $\mu g\,L^{-1}$ respectively. Similarly to individual compounds concentrations, the bioassays equivalent concentrations were already below the guidelines values in the water entering the reclamation plant. Final effluent median equivalent concentrations were also several orders of magnitude below the corresponding guideline values, i.e. more than 2900, 33 and 428 fold for estrogenicity, neurotoxicity and phytotoxicity respectively.

For the parameters considered, the water quality complies with the requirements of the Australian Guidelines for Water Recycling: Augmentation of Drinking Water Supplies. This suggests that such a treatment train could be considered as an alternative to the combination of microfiltration and reverse osmosis for indirect potable reuse schemes. It has the advantage of not producing a waste stream and would be certainly less energy intensive. Nevertheless, before this process can be recommended for indirect potable reuse, additional consideration needs to be given to the overall risk management strategies of the treatment train. Moreover, the removal of pathogens such as viruses and bacteria has to be assessed as well as the potential to form disinfection by-products due to the remaining DOC levels.

4. Conclusions

The assessment of a tertiary treatment train regarding the removal of micropollutants and decrease of biological activity leads to the following conclusions:

1) The treatment train reduced the concentration of the 54 micropollutants quantified in the secondary treated wastewater as well as the biological activity observed in bioassays. Overall concentration reductions were typically higher than 90% and most of the compounds were removed to levels lower than 0.01 $\mu g\,L^{-1}$. The observed effects decreased by 62% to more than 90% depending on the assay and levels in the final effluent were close to or equivalent to the blank's. The effect of individual processes varied from one compound and bioassay to another but the combination of the coagulation/flocculation/DAFF stage, the main ozonation and the activated carbon filtration was responsible for the major part of the observed reduction.
2) The use of a battery of bioassays as complementary tools to chemical analysis yielded valuable additional information on the water quality and the process efficiency. The results showed the limitations of chemical analysis to assess potential biological adverse effects and the ability of bioassays to take into account the presence of non-measured compounds, formed transformation products and/or mixture effects.
3) Concurrent measurement of DOC, micropollutants and biological activity showed that the DOC level can influence the effect observed in the bioassays. Baseline toxicity was shown to correlate well with the DOC levels. It is supposed that non-targeted organic matter is co-extracted with the targeted organic micropollutants in the SPE purification step. More understanding of the nature and quantity of these co-extracted compounds is needed.
4) Ozone dose relative to DOC content is a key parameter for the ozonation performance. A low ozone dose of 0.1 $mg_{O3}\,mg_{DOC}^{-1}$ did not affect the concentration of micropollutants or biological activity whereas a dose of 0.5 $mg_{O3}\,mg_{DOC}^{-1}$ was able to remove most of the micropollutants by more than 70% and substantially decrease the biological activity.
5) Degradation products are a major concern in ozonation processes. Here, the results from the battery of bioassays show that the main ozonation leads to lower baseline and specific toxic effects. It can be concluded that the mixture of degradation products formed have an overall less harmful potential than the mixture of parent compounds.

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Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.watres.2009.09.048.

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