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Evaluating the efficiency of advanced wastewater treatment: Target analysis of organic contaminants and (geno-)toxicity assessment tell a different story☆

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ABSTRACT

At a pilot scale wastewater treatment plant ozonation and powdered activated carbon filtration were assessed for their efficacy to remove trace organic contaminants from secondary treated effluents. A chemical analysis of 16 organic compounds was accompanied by a comprehensive suite of *in vitro* and *in vivo* bioassays with the focus on genotoxicity to account for the potential formation of reactive oxidation products. In vitro experiments were performed with solid phase extracted water samples, *in vivo* experiments with native wastewater in a flow through test system on site at the treatment plant. The chemical evaluation revealed an efficient oxidation of about half of the selected compounds by more than 90% at an ozone dose of 0.7 g/g DOC. A lower oxidizing efficiency was observed for the iodinated X-ray contrast media (49–55%). Activated carbon treatment (20 mg/L) was less effective for the removal of most pharmaceuticals monitored. The *umuC* assay on genotoxicity delivered results with about 90% decrease of the effects by ozonation and slightly lower efficiency for PAC treatment. However, the Ames test on mutagenicity with the strain YG7108 revealed a consistent and ozone-dose dependent increase of mutagenicity after wastewater ozonation compared to secondary treatment. Sand filtration as post treatment step reduced the ozone induced mutagenicity only partly. Also the fish early life stage toxicity test revealed an increase in mortality after ozonation and a reduced effect after sand filtration. Only activated carbon treatment reduced the fish mortality compared to conventional treatment on control level. Likewise the *in vivo* genotoxicity detected with the comet assay using fish erythrocytes confirmed an increased (geno-)toxicity after ozonation, an effect decrease after sand-filtration and no toxic effects after activated carbon treatment.

This study demonstrates the need for a cautious selection of methods for the evaluation of advanced (oxidative) treatment technologies and of the effectiveness of post-treatments for elimination of adverse effects caused by oxidative treatments case by case.

☆ This paper is dedicated to the memory of our colleague Dr. Thomas Knacker, a much valued research partner, friend and pioneer of tight coupling between analytical chemistry and ecotoxicology.

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1. Introduction

The significance of reducing wastewater (WW) associated organic contaminants by upgrading WW treatment plants (WWTP) and its relevance for the aquatic ecosystem has recently been discussed (e.g., Joss et al., 2008; Englert et al., 2013; Stalter et al., 2013). Powdered activated carbon (PAC) adsorption and ozonation of secondary effluents are intended for feasible advanced treatment technologies (Joss et al., 2008) and have proven to further remove or degrade anthropogenic organic contaminants (Ternes et al., 2003; Westerhoff et al., 2005).

WW represents a complex mixture that contains of a huge number of chemicals. Advanced WW treatment technologies alter that mixture in different ways. As an adsorptive process PAC effectively removes substances, while ozonation mainly transforms organic contaminants into – predominantly – unknown oxidation products (OPs) with unknown toxicity (Joss et al., 2008; Stadler et al., 2012) or, as the best case, mineralizes them (Klavarioti et al., 2009). The number of studies elucidating oxidation pathways of single organic pollutants is growing constantly (e.g., Huber et al., 2004; Benner and Ternes, 2009; Bourgin et al., 2013). The formation of one major fraction of the total OPs – so called byproducts (small organic molecules: assimilable organic carbon (AOC), aldehydes, carboxylic acids, ketones) – has been well described (e.g., Richardson et al., 1999; Wert et al., 2007). Depending on the applied ozone dose and the dissolved organic carbon (DOC) content this fraction of OPs may reach concentrations up to the low mg/L range. Even if considered as biodegradable, it is in question if half-life of the byproducts in surface waters is short enough and its toxicity low enough to be not harmful to aquatic ecosystems. Furthermore, some of known byproducts, e.g., methanal, glyoxal, and the OPs of metoprolol and thiamethoxam are genotoxic (Kuchenmeister et al., 1998; Sojic et al., 2012). Following Stadler et al. (2012) it is unrealistic to describe all transformation products (TPs) and their toxicity considering 10,000s of potential parent compounds that might be present in WW. Besides the control of removal efficiency of key substances it is crucial to evaluate advanced treatments with appropriate toxicity tests, which cover a broad range of endpoints and in particular endpoints that are likely to detect effects of TPs formed in the respective treatment process, e.g., reactive toxicity endpoints in case of oxidation processes. The latter is critical to evaluate potential risks posed by TPs formed during advanced treatment steps. However, decisive for the outcome of toxicity tests is sample pretreatment and enrichment. Any sample enrichment step leads to the loss of water constituents (Daughton, 2003) and in particular oxidation products might be poorly extractable (Benner and Ternes, 2009). Accordingly, using WW samples that are nearly unchanged compared to the original effluent composition (e.g., low storage time, no alteration by sample preparation or enrichment) is crucial for a comprehensive risk-benefit evaluation.

In the present study analytical measurements of selected compounds as process control are combined with toxicological data of chronic *in vivo* and *in vitro* tests with focus on genotoxicity for the evaluation of ozonation and PAC addition as advanced treatments.

2. Material & methods

2.1. Pilot plant setup

The investigated pilot WWTP received wastewater from the primary clarifier of the municipal WWTP Neuss-South (population equivalent 120,000; 13,500 m^3/d; Neuss, Germany). At the pilot plant the wastewater was processed with conventional biological activated sludge treatment (Fig. 1A). After secondary settling an effluent ozonation was performed (0.7 g O_3/g DOC, contact time 18 min) followed by a sand filtration step after the ozone reactor (empty bed contact time (EBCT) 40 min, Fig. 1C). In parallel, powdered activated carbon (PAC, Norit SAE Super; Cabot Norit, Amersfoort, The Netherlands, Table S10) treatment was tested (20 mg/L, contact time 60 min) after conventional treatment with subsequent sand filtration (EBCT 3 h, Fig. 1B). The sand filters were installed as a three layer filter system in a 2 m glass column consisting of an anthracite layer on top followed by a sand layer and a closing filter gravel layer. The sand filtration systems started operating four weeks prior to test initiation to allow microorganism colonization. A filter back-flush was performed at every 2nd day. Due to the long test duration only one ozone and one PAC dose could be applied for the *in vivo* toxicity experiments. However, these doses have already been proven to efficiently remove or oxidize organic contaminants effectively (Nowotny et al., 2007; Hollender et al., 2009) and are regarded as economically feasible (Joss et al., 2008). More information about the pilot plant setup can be found in the supplement. WW quality parameters after the conventional biological treatment are provided in Table 1.

Low ammonium and nitrate concentrations after final sedimentation indicated that the pilot treatment plant was working in an appropriate manner (Hoen et al., 1996). In total, four different sampling points of serial or parallel treatment steps were monitored and tested as illustrated in Fig. 1.

2.2. Chemical analysis

2.2.1. Sample preparation for chemical analysis

Flow proportional 24 h composite samples were filtered through glass fiber filters (GF 6, <1 μm, diameter 55 mm from Schleicher and Schuell, Dassel, Germany). For solid phase extraction (SPE) of water samples, 200 mL of water were spiked with the respective surrogate standards. Different SPE methods were used for the extraction of acidic pharmaceuticals, iodinated X-ray contrast media, antibiotics and psychoactive drugs, all SPE cartridges were pre-conditioned with 1 × 2 mL n-heptane, 1 × 2 mL acetone, 3 × 2 mL methanol followed by 4 × 2 mL water. The water samples were passed through the pre-conditioned SPE cartridges at a flow rate of approximately 10 mL/min. Subsequently, the solid-phase material was dried completely with a commercially available drying device (Grenzahl-Wyhlen, Germany) by a continuous nitrogen stream for 1 h.