



Formation of carbonyl compounds during ozonation of lake water and wastewater: Development of a non-target screening method and quantification of target compounds

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ABSTRACT

Ozonation of natural waters is typically associated with the formation of carbonyl compounds (aldehydes, ketones and ketoacids), a main class of organic disinfection byproducts (DBPs). However, the detection of carbonyl compounds in water and wastewater is challenged by multiple difficulties inherent to their physicochemical properties. A non-target screening method involving the derivatisation of carbonyl compounds with *p*-toluene-sulfonylhydrazine (TSH) followed by their analysis using liquid chromatography coupled to electrospray ionisation high-resolution mass spectrometry (LC-ESI-HRMS) and an advanced non-target screening and data processing workflow was developed. The workflow was applied to investigate the formation of carbonyl compounds during ozonation of different water types including lake water, aqueous solutions containing Suwannee River Fulvic acid (SRFA), and wastewater. A higher sensitivity for most target carbonyl compounds was achieved compared to previous derivatisation methods. Moreover, the method allowed the identification of known and unknown carbonyl compounds. 8 out of 17 target carbonyl compounds were consistently detected above limits of quantification (LOQs) in most ozonated samples. Generally, the concentrations of the 8 detected target compounds decreased in the order: formaldehyde > acetaldehyde > glyoxylic acid > pyruvic acid > glutaraldehyde > 2,3-butanedione > glyoxal > 1-acetyl-1-cyclohexene. The DOC concentration-normalised formation of carbonyl compounds during ozonation was higher in wastewater and SRFA-containing water than in lake water. The specific ozone doses and the type of the dissolved organic matter (DOM) played a predominant role for the extent of formation of carbonyl compounds. Five formation trends were distinguished for different carbonyl compounds. Some compounds were produced continuously upon ozonation even at high ozone doses, while others reached a maximum concentration at a certain ozone dose above which they decreased. Concentrations of target and peak areas of non-target carbonyl compounds during full-scale ozonation at a wastewater treatment plant showed an increase as a function of the specific ozone dose (sum of 8 target compounds ~ 280 µg/L at 1 mgO₃/mgC), followed by a significant decrease after biological sand filtration (> 64–94% abatement for the different compounds). This highlights the biodegradability of target and non-target carbonyl compounds and the importance of biological post-treatment.

1. Introduction

The efficiency of ozone as an oxidant and disinfectant, along with concerns about the formation of halogenated disinfection byproducts (DBPs) during chlorine-based processes, made ozonation a good alternative for oxidative water treatment (von Sonntag and von Gunten 2012). Ozonation was introduced as a disinfection step in drinking water

at the beginning of the twentieth century (von Sonntag and von Gunten 2012). More recently, ozonation has been increasingly applied in wastewater treatment for the abatement of organic micropollutants, known to induce a spectrum of adverse effects to aquatic ecosystems (Eggen et al., 2014; Hollender et al., 2009; Joss et al., 2008; Kienle et al., 2022; Ternes et al., 2003). Furthermore, ozonation is increasingly considered as an oxidation step in water reuse treatment trains (Gerrity

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et al., 2013; von Gunten 2018).

Similar to other oxidants/disinfectants, the application of ozone to achieve different water and wastewater treatment goals is accompanied by the formation of DBPs due to reactions with matrix components, such as bromide and dissolved organic matter (DOM) (Lim et al., 2022; Manasfi 2021; Richardson and Ternes 2022; von Gunten 2003; von Gunten 2018; von Sonntag and von Gunten 2012). Bromate is the most critical inorganic DBP formed during ozonation because of its possible carcinogenicity, and it is regulated in drinking water (World Health Organization 2017). Amongst organic DBPs formed during ozonation, carbonyl compounds including aldehydes, ketones, oxo- and keto-acids are key classes.

The formation of carbonyl compounds is well documented during ozonation of natural organic matter (NOM) isolates and drinking water (Hammes et al., 2006; Huang et al., 2005; Liu et al., 2020; Swietlik et al., 2004; Weinberg et al., 1993). However, there is only limited information on the formation of carbonyl compounds during ozonation of wastewater (Marron et al., 2020; Wert et al., 2007).

Overall, about 50 carbonyl compounds have so far been proposed to be formed during ozonation (Hammes et al., 2006; Huang et al., 2005; Marron et al., 2020; Richardson et al., 2000; Richardson et al., 1999; Swietlik et al., 2004; Tentscher et al., 2018; Weinberg and Glaze 1996; Wert et al., 2007). Some carbonyl compounds (e.g. benzoquinones and α,β -unsaturated dialdehydes/ketones) were not detected in water treatment but rather in ozonation studies with model compounds (Tentscher et al., 2018; Zoumpoulis et al., 2021). Considering the diversity of potential carbonyl precursors in DOM, the currently known carbonyl compounds represent only a small fraction of the universe of this compound class.

The main concern about the formation of carbonyl compounds is related to their toxicity, which is induced by their electrophilic nature. This allows them to form covalent bonds with nucleophilic moieties of biological macromolecules such as enzymes, proteins, and DNA (LoPachin and Gavin 2014; Prasse 2021; Prasse et al., 2018). Exposure to α,β -unsaturated carbonyl compounds has been associated with development of cardiovascular and neurological diseases, and diabetes (LoPachin and Gavin 2014). In addition, carbonyl compounds can possibly catalyse the formation of other potentially toxic byproducts such as nitrosamines (Lv et al., 2009) and can act as precursors for halogenated byproducts during post-chlorination (Barrott 2004; Marron et al., 2021; Weinberg and Glaze 1996). The formation of carbonyl compounds also contributes to the assimilable/biodegradable organic carbon, which may promote microbial (re-)growth after ozonation (Hammes et al., 2006; Zimmermann et al., 2011). Finally, often an increased toxicity is observed after ozonation of secondary wastewater effluent, which is in many cases abated during biological post-filtration (Magdeburg et al., 2014; Stalter et al., 2010; Volker et al., 2019). The latter points towards easily biodegradable substances and carbonyl compounds may explain this observation to some extent (von Gunten 2018).

In the US, a maximum contaminant level of 50 $\mu\text{g/L}$ for total aldehydes and a notification level of 100 $\mu\text{g/L}$ for formaldehyde in drinking water have been introduced in the states of New York and California, respectively (California Division of Drinking Water State Water Resources Control Board 2020; New York State Department of Health, 2021). However, no standards for carbonyl compounds have been established by the European Union and the World Health Organization, since typical concentrations in drinking water are considered well below concentrations of health concern (European Commission 2020; World Health Organization 2022).

The analysis of carbonyl compounds faces multiple challenges related to their physicochemical properties. Carbonyl compounds are generally very polar with low molecular weights (MW) resulting in poor separation on reverse-phase liquid chromatography (LC) columns. They are typically poorly ionisable in electrospray ionisation (ESI) and have a low detectability by mass spectrometry. To address these challenges, carbonyl compounds are typically derivatised before analysis.

Derivatisation methods involve O-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine (PFBOA) (Glaze et al., 1989), pentafluorobenzylhydroxylamine (PFBHA) (Richardson et al., 1999), and 2,4-dinitrophenylhydrazine (DNPH) (Richardson et al., 2000). These methods require laborious sample preparation, as they typically involve liquid-liquid or solid-phase extraction, and in the case of DNPH suffer from its limited solubility in aqueous solutions and tendency to deposit in the MS ion-source (Siegel et al., 2014). Alternatively, a derivatisation method based on *p*-toluenesulfonylhydrazine (TSH) has been applied for the screening of carbonyl compounds in biological samples (Siegel et al., 2014) or for the targeted analysis of selected carbonyl compounds in water reuse systems (Marron et al., 2020). However, a systematic assessment of the TSH method performance in water and wastewater matrices has not been performed so far, and the method has only been applied for targeted analysis of selected carbonyl compounds.

Therefore, the present study aimed at (i) developing, optimizing and validating an analytical method based on TSH derivatisation and high-resolution mass spectrometry (HRMS) for the non-targeted screening and targeted quantification of carbonyl compounds, (ii) determining their concentrations and formation trends in DOM-containing synthetic water, wastewater and lake water, and (iii) monitoring their fate during full-scale ozonation of a secondary wastewater effluent followed by biological post-treatment.

In a companion study, unknown carbonyl compounds are detected and partially identified by the method from this paper along with ozonation of model compounds for mechanistic assessments (Houska et al., 2023).

2. Materials and methods

2.1. Reagents, stock solutions, and chemicals

A list of reagents and solutions used in this study is provided in Section S1 in the Supporting Information (SI). All aqueous solutions were prepared in ultrapure water with a resistivity of $> 18.2 \text{ M}\Omega\cdot\text{cm}$, ASTM Type 1 from arium® pro Ultrapure Laboratory Water Systems, Sartorius.

In total, 17 different target carbonyl compounds were selected including saturated and unsaturated aldehydes, ketones, and keto-acids (Fig. 1). Further information on the target carbonyl compounds (purity, CAS number, molecular formula and supplier) is provided in Table S1 (SI).

2.2. Sample collection, preparation and ozonation

Lake water samples were collected from lakes Greifensee and Lac de Bret, Switzerland. Grab secondary wastewater effluent samples were collected from three wastewater treatment plants (WWTPs): Neugut in Dübendorf, Werdhölzli in Zurich, and Glarnerland in Bilten, Switzerland. At the WWTP Werdhölzli, three sampling campaigns were carried out on different dates (September 2020, March 2021, and October 2021). During the March 2021 sampling campaign, grab samples were also collected after full-scale ozonation (O_3 plant) at three different ozone doses (Table S2 (SI)). Samples corresponding to the same water package from the ozonated effluent were also collected after post-treatment by biological sand filtration. Samples were collected in pre-cleaned glass bottles, and were filtered in the laboratory using pre-rinsed $0.45 \mu\text{m}$ glass fibre filters (Sartorius AG, Göttingen, Germany) within a few hours after sampling. Laboratory-scale ozonation was performed by spiking samples with an ozone stock solution (Section S1, SI). To assess the potential influence of filtration of Werdhölzli WWTP effluent on the formation of carbonyl compounds during laboratory-scale experiments, ozonation of a sample (March 2021 sampling campaign) was carried out with filtered and unfiltered water. Samples were then stored at 4°C until analysis. Water quality parameters including dissolved organic carbon (DOC) concentrations are provided

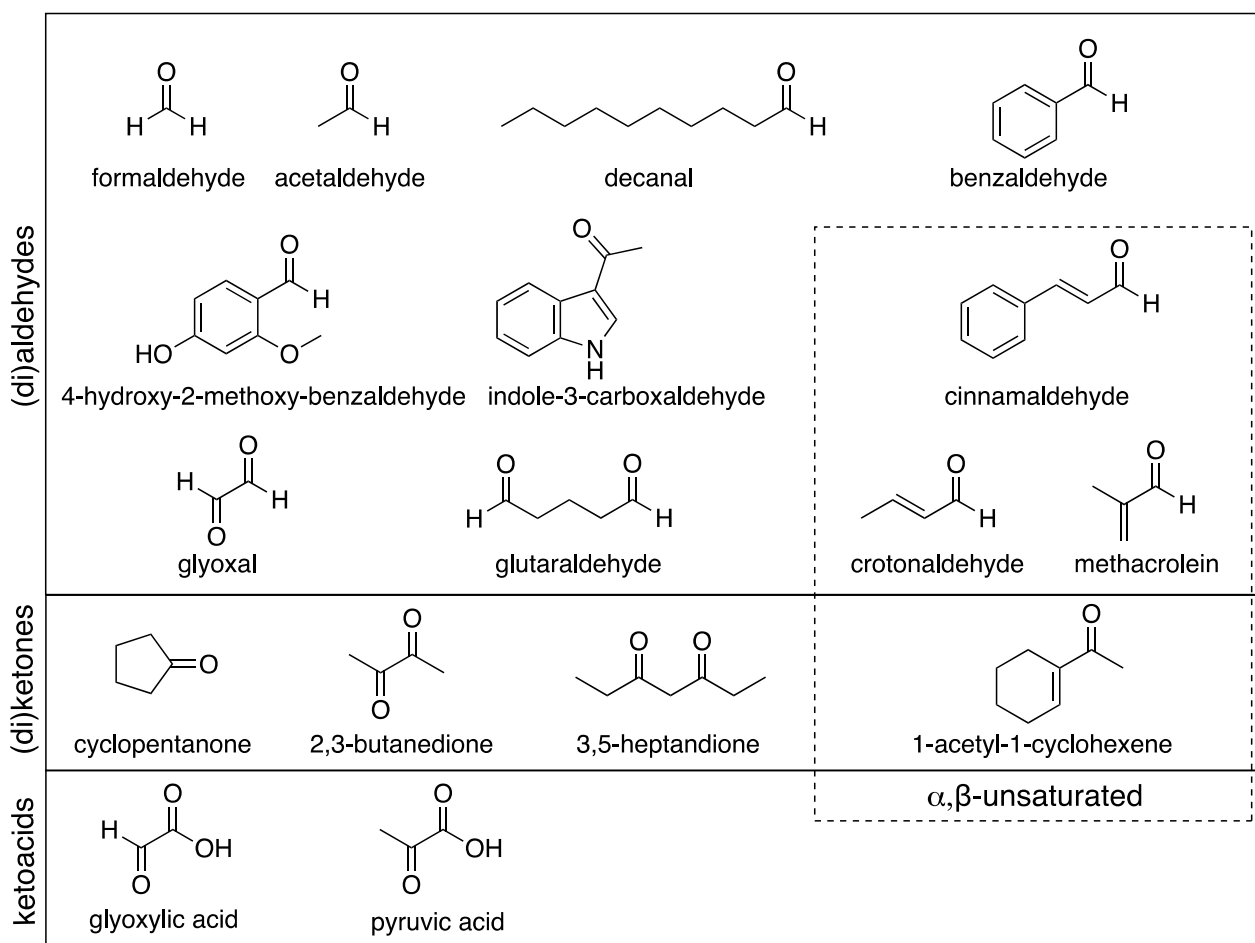


Fig. 1. Structures and chemical classes of the selected target carbonyl compounds.

in Table S2 (SI). Model DOM samples (DOC concentration 5 mg/L) were prepared using a stock solution of Suwannee River II Standard Fulvic Acid (SRFA) isolate (50 mgC/L) in a phosphate buffer (5 mM, pH 7). Lake water, secondary wastewater effluent samples (referred to as wastewater), and SRFA samples were treated with multiple specific ozone doses using an ozone stock solution (1.5–1.9 mM), Section S1 (SI).

2.3. Derivatisation procedure and analysis by LC-ESI-HRMS

The derivatisation reaction of carbonyl compounds was carried out using TSH in the presence of HCl as a catalyst (Fig. 2). This is a nucleophilic addition of hydrazine (TSH) to the carbonyl group, resulting in the formation of a hydrazone and H₂O (Fig. 2). Details on the optimisation of the derivatisation conditions and the calculation of derivatisation efficiency are provided in Section S3 (SI). Different derivatisation conditions including various TSH concentrations (Figures S2 and S3, SI), HCl concentrations (Figure S4a, SI), and reaction times (Figure S4b, SI) were tested for the derivatisation of carbonyl compounds in different water types.

Samples were spiked with benzaldehyde-d₆ (100 nM) and tramadol-d₆ (32.5 nM), as derivatisation surrogate and internal standard, respectively. The samples were then derivatised by the addition of TSH (200 μ M) and HCl (0.02 M). A flow scheme of the different steps during derivatisation is presented in Figure S1 (SI). Derivatisation of samples was performed within 24 h after collection or generation.

The samples were analysed using high-performance LC (UltiMate 3000 UHPLC system, Dionex) coupled to an orbitrap HRMS (Q Exactive Plus, Thermo Scientific) with ESI. Separation was performed using an Atlantis column (3 μ m particle size, 3 \times 150 mm) at 30 °C and a flow rate

of 300 μ L/min. An autosampler (PAL HTS-xt system, McKinley scientific) was used for sample injection (50 μ L sample injected into a 100 μ L loop) and for sample storage at 4 °C during measurement sequences. Eluents consisted of ultrapure water (A) and methanol (B) each containing 0.1% formic acid. The LC method ran for 29 min and the LC gradient started with 95% A. After 1 min, B increased gradually from 5% to reach 95% at 17 min and stayed constant until 25 min. Afterwards, A increased to reach 95% (starting gradient) at 27 min and then remained constant until the end of the run. During the first 5 min of the run, the LC flow was diverted to waste to avoid contamination of the MS with phosphate buffer, which was present in most samples, and the ESI spray voltage was meanwhile set to 0 V. Moreover, the intense peak of the derivatising agent (RT: 11.9 min) was cut out by diverting the LC flow to waste between about 11.8 and 12.3 min to avoid any contamination of the MS. An ultrapure water blank was injected after each derivatised sample to avoid potential carry-over of derivatised carbonyls or TSH from one sample to another.

Acquisition was achieved by performing an initial MS full-scan (resolution 140 000 at m/z 200) followed by data-dependant fragmentation MS² experiments (resolution 17 500 at m/z 200) in the positive polarity mode. The latter was selected since it provided better detection of the derivatised carbonyls than the negative polarity mode (Figure S5, SI).

MS conditions were as follows: spray voltage 4000 V, capillary temperature 350 °C, sheath gas 40 arbitrary units, auxiliary gas 10 arbitrary units, and mass range from 100 to 1000 Da. Properties of the data-dependant (top 5) MS² scans included an Automatic Gain Control (AGC) of 2×10^5 , isolation window of 1.0 m/z , and normalised collision energy (NCE) of 30. The 17 derivatised target carbonyls in positive ESI

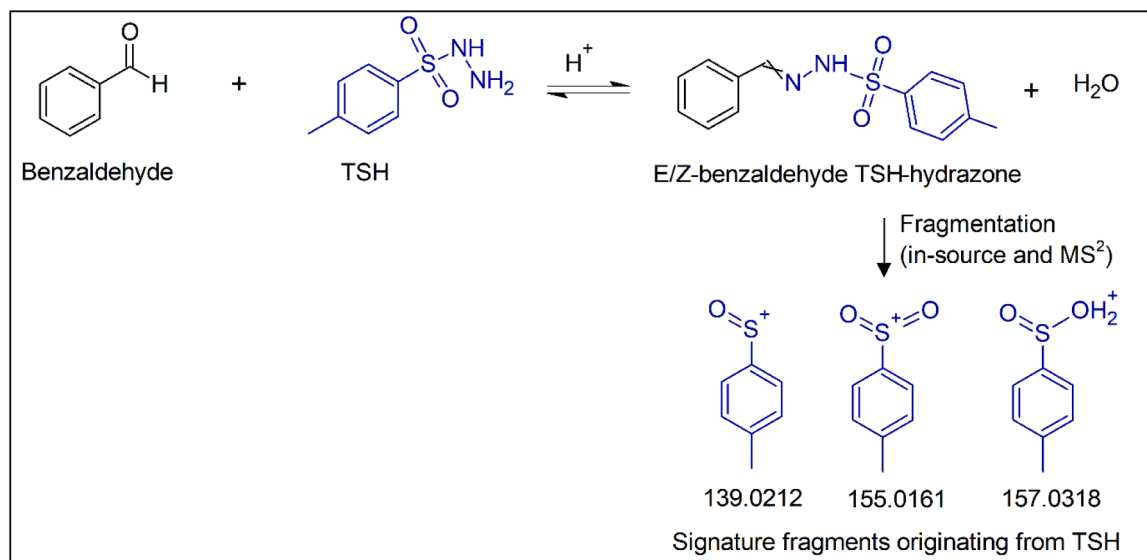


Fig. 2. Acid-catalysed TSH derivatisation of a carbonyl compound by a nucleophilic attack of the hydrazine on the carbonyl (benzaldehyde is shown as an example) and formation of the corresponding hydrazone. The fragmentation of the formed hydrazone during MS^2 resulting in the three TSH signature fragments, which were used for the identification of carbonyl compounds in the non-target workflow, is also shown.

were added to an inclusion list to ensure their fragmentation.

2.4. Method performance characteristics, matrix effects, and stability of carbonyl compounds

The concentrations of target carbonyl compounds were quantified based on external calibrations, since matrix effects were negligible (Table S4, SI). Calibration standards were prepared by spiking carbonyl compounds into ultrapure water followed by derivatisation. The response ratio, defined as the ratio of peak area of an analyte to the peak area of TSH-derivatised benzaldehyde- d_6 in the same sample, was used for calibration and quantification. The limits of detection (LOD) and limits of quantification (LOQ) were defined based on a signal-to-noise (S/N) ratio of 3:1 and 10:1, respectively. The calibration ranges were defined based on the linearity of calibration curves and the coverage of concentrations measured in the samples.

Potential interferences on the response of carbonyl compounds in ESI-HRMS due to matrix effects were assessed according to the protocol presented in Section S4 (SI). In addition, the stability of carbonyl compounds during storage at 4 °C in derivatised and non-derivatised forms was assessed (for details see Section S5 (SI)).

2.5. Non-target screening and data processing workflow

A non-target screening workflow was implemented using the software Compound Discoverer (CD3.2, Thermo Scientific, Germany). The workflow consisted of an array of nodes used for the processing and post-processing of LC-MS data. The detection of TSH-derived fragments (referred to as signature fragments) in the MS^2 spectra was used as a key criterion for the screening of carbonyl compounds (Fig. 2). A detailed description of the non-target workflow and data processing is presented in Section S6 (SI). A total of 178 carbonyl compounds were detected in all samples (Houska et al., 2023). Among the latter, 40 non-target carbonyl compounds were prioritized for monitoring because they occurred in more than two water types and contained ≤ 5 carbons (with one exception for a C_6 compound that was also included). The Trace-Finder Software (TF5.1, Thermo Scientific, Germany) was used for the determination of peak areas of the 40 non-target carbonyl compounds and concentrations of target compounds.

3. Results and discussion

3.1. Optimisation of the derivatisation procedure in the different water types

The main parameters for the optimisation/validation of the derivatisation procedure are discussed here, with additional details in Section S3 (SI).

3.1.1. TSH concentration

The variability of derivatisation efficiency as a function of the TSH concentration was similar for the target carbonyl compounds across all tested matrices. A TSH concentration of 200 μ M provided the highest derivatisation efficiency (Figures S2 and S3, SI).

3.1.2. HCl concentration

The derivatisation efficiencies increased considerably in the presence of HCl (≥ 0.01 M), except for formaldehyde (no effect), acetaldehyde (decreasing efficiency) and decanal (slightly decreasing efficiency) (Figure S4a, SI). Increasing HCl concentrations from 0.01 to 0.05 M did not enhance the derivatisation efficiency, and therefore, a HCl concentration of 0.02 M was selected to overcome potential buffering capacities in wastewater.

3.1.3. Reaction time

Changes in the derivatisation efficiencies were minor (within 10%) for most tested carbonyl compounds as the time interval between derivatisation and analysis increased from 10 min to 2 h (Figure S4b, SI). Therefore, a short reaction time of 10 min after the addition of TSH and HCl was sufficient to achieve maximum derivatisation.

The application of the optimised derivatisation method significantly enhanced the detectability of most target carbonyl compounds. While some compounds were also detectable before derivatisation, most compounds became detectable only after derivatisation (Figure S5, SI).

3.2. Method performance characteristics and stability of the target carbonyl compounds

Method performance characteristics including the LODs, LOQs and measurement ranges are presented in Table S3 (SI). In addition, the results of the assessment of analytical matrix effects during the analysis

of carbonyl compounds in water and wastewater matrices are presented in Section S4 and Table S4 (SI). Based on the determined LODs (0.004–1.35 µg/L, 0.06–13.5 nM), the sensitivity of the TSH method was generally better or in a similar range to the sensitivity achieved by the PFBOA and DNPH derivatisation methods, which involve extensive sample preparation such as longer derivatisation times (1–4 h) and extraction steps and provide LODs in the range of 0.7–5.2 µg/L and 0.5–1 µg/L, respectively (Glaze et al., 1989; Richardson et al., 2000). Siegel et al. (2014) also reported generally lower LODs for carbonyl compounds with the TSH method compared to the DNPH method for biological samples (cell extracts). Formaldehyde was an exception, with a markedly higher LOD (4.50 µg/L or 150.2 nM) than for other carbonyl compounds and other derivatisation methods due to high background levels detected in TSH blanks. The source of background formaldehyde concentration was attributed to TSH, and it was accounted for in the determination of formaldehyde concentrations (Section S4, SI). However, since the formation of formaldehyde is typically high during ozonation, this limitation is not critical in the context of the present study.

Matrix effects were negligible during the analysis of derivatised carbonyl compounds in both water and wastewater (Table S4, SI), which agrees with previous findings in cell extracts (Siegel et al., 2014).

The stability of the target carbonyl compounds before and after TSH derivatisation is presented in Section S5 (SI). In the TSH-derivatised form, a broad disparity in the stability of carbonyl compounds in wastewater was observed over a storage period of 6.5 days at 4 °C (Figure S6, SI). Derivatised carbonyl compounds were either highly unstable (i.e., > 40% loss within 24 h and declined to < LOQ after 6.5 days, e.g., glyoxal, glyoxylic acid, pyruvic acid, and formaldehyde), moderately unstable (i.e., < 65% loss within 6.5 days, e.g., decanal, crotonaldehyde and methacrolein) (Figure S6a, SI), or quite stable (i.e., < 10% loss within 6.5 days, e.g., 1-acetyl-1-cyclohexene, acetaldehyde,

2,3-butanedione, glutaraldehyde, and 3,5-heptanedione) (Figure S6b, SI). In the non-derivatised form, some carbonyl compounds tended to degrade (e.g., glutaraldehyde, pyruvic acid, glyoxylic acid), while others appeared during storage (e.g., glyoxal) (Figure S7, SI). This assessment emphasizes the importance of performing the derivatisation and analyses immediately after sampling for the detection and accurate determination of concentrations of carbonyl compounds.

3.3. Identification of carbonyl compounds by the non-target screening workflow

The application of the non-target screening workflow allowed to effectively reduce the complexity of HRMS data in complex matrices such as wastewater, and to identify unknown carbonyl compounds. The workflow applied in this study is presented in Fig. 3 for a plant-ozonated secondary wastewater effluent as an example. Further details on the workflow are provided in Section S6 (SI).

The workflow for identification of compounds was based on the acquired MS and MS² spectra ((1) in Fig. 3) where accurate masses (error within 5 ppm) were determined. The chemical formulas were assigned using CD3.2 based on the accurate mass, isotope patterns, and fragmentation in MS² ((2) in Fig. 3, see Figure S8 and Section S6 (SI) for further information). Filtering of the identified compounds was achieved by excluding detected compounds which occurred in the derivatised blank at an intensity above 20% compared to the sample ((3) in Fig. 3). For the plant-ozonated wastewater provided as an example in Fig. 3, this background subtraction reduced the number of potential compounds by > 40% (number of hits decreasing from 1734 to 978). The secondary filtering criteria consisted of excluding compounds that did not have the minimal composition of C₈H₁₀N₂O₂S, which results from derivatisation of carbonyl compounds and those with a RT lower than that of TSH ((4) in Fig. 3). For the example in Fig. 3, this reduced

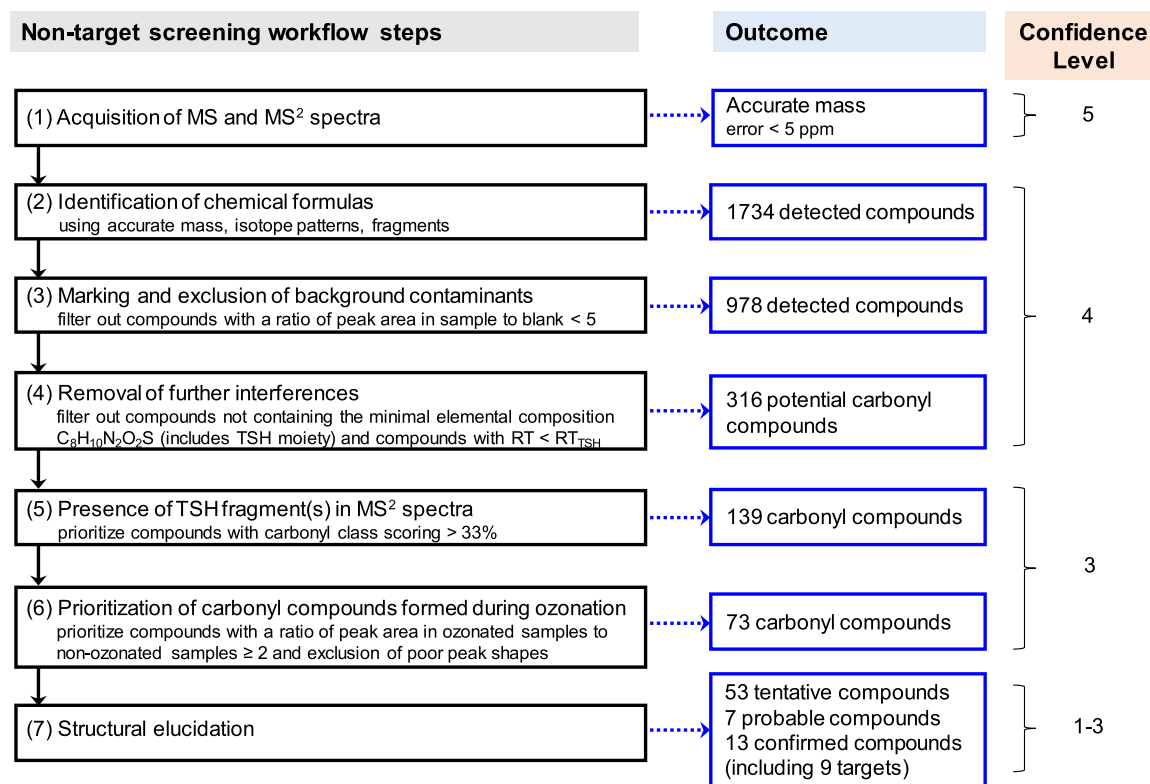


Fig. 3. Application of the non-target workflow and prioritisation criteria resulting in the identification of unknown carbonyl compounds at confidence levels from 1 to 3 (Schymanski et al., 2014). Actual numbers are shown for a plant-ozonated secondary wastewater effluent at the Werdhölzli WWTP as an example. Elucidation of structures involving ozonation of model compounds, amongst other criteria, is presented in Houska et al. (2023).

the number of hits by > 60% to 316 potential carbonyl compounds. The exclusion of compounds with an $RT < RT_{TSH}$ was based on the assumption that the binding of an additional organic moiety (carbonyl compound) to TSH leads to a final product with an enhanced retention in reverse-phase chromatography (C_{18} column). Therefore, TSH-derivatized carbonyls will have a $RT \geq RT_{TSH}$, which was confirmed for all the tested target carbonyl compounds. A further filtration criterion excluded compounds for which the MS^2 spectra did not contain any of the three TSH signature fragments shown in Fig. 2 (compound class score < 33%) and which were considered most likely as non-carbonyl-containing compounds ((5) in Fig. 3 and Section S6, SI). The exclusion of such compounds reduced the number of hits by 56% to 139 compounds. Furthermore, from the 139 hits, only the compounds which occurred at \geq two-fold intensity in the ozonated sample and had satisfying peak shapes were processed. Consequently, the number of hits was reduced by 47% to a pool of 73 compounds ((6) in Fig. 3). Overall, the combination of the four filtering criteria allowed the screening and identification of carbonyl compounds with low risk of false positives and reduced the pool of compounds under consideration by about 96%. To determine the chemical formula of a carbonyl compound (in the non-derivatised form), $C_7H_8N_2OS$ or $C_{14}H_{16}N_4O_2S_2$ (in case of double derivatised dicarbonyl compounds) were subtracted from the identified chemical formula of the derivatised compound using the scripting node (Section S6, SI). This allowed to assign chemical formulas for the non-derivatised carbonyl compounds. After these processing steps, the identification of carbonyl compounds was at confidence level (CL) = 3, given the presence of a carbonyl functional group, according to the classification by Schymanski et al. (2014). Structural elucidation was performed in step 7 ((7) in Fig. 3) where tentative, probable, and confirmed identifications were achieved by integrating analytical and mechanistic data (including from the ozonation of model compounds), as described in Houska et al. (2023). For the 73 compounds filtered in the plant-ozonated wastewater sample ((6) in Fig. 3), 53 tentative (CL = 3), 7 probable (CL = 2), and 4 confirmed (CL = 1) structures were elucidated, along with 9 additional confirmed compounds which matched compounds present in the target list ((7) in Fig. 3). Overall, the non-target workflow allowed the identification of the molecular formulas of 178 carbonyl compounds (number of carbons ranging from C: 1–18) in the different water types. The 178 detected carbonyl compounds included 166 unknowns and 12 targets (with 4 compounds detected only < LOQ). The 166 unknown carbonyl compounds were identified as follows: 151 tentative compounds (CL = 3), 7 probable compounds (CL = 2), and 8 confirmed compounds (CL = 1), as described in detail in Houska et al. (2023). The detected target compounds are described in Section 3.4.

The validation of the workflow was achieved based on the identification of the target carbonyl compounds, which were treated as unknowns for testing. Applying the workflow on the target carbonyls (using standard solutions within the concentration range reported in Table S3 (SI)), 14 out of 17 target carbonyl compounds could be identified when processed as unknowns. The three target carbonyl compounds which escaped detection (1-acetyl-1-cyclohexene, indole-3-carboxaldehyde, and glyoxylic acid) were excluded due to the absence of the TSH signature fragments in their MS^2 spectra or occurrence at intensities below the detection threshold. The assignment of three target carbonyl compounds as false negatives is caused by the conservative nature of the applied non-target approach which aimed at providing high specificity in detecting carbonyl compounds to avoid false positives. While this approach has an advantage of providing high confidence in the assignment of carbonyl-containing compounds, its drawback is the potential exclusion of carbonyl compounds presenting MS^2 spectra with no or low TSH fragments intensity. However,

considering the high number of carbonyl compounds detected despite the restrictions in the non-target workflow and the requirement of further prioritisation to achieve a manageable pool of compounds for structural elucidation (Houska et al., 2023), the workflow can be considered fit for purpose.

3.4. Formation of target carbonyl compounds during ozonation in laboratory-scale experiments

3.4.1. Relative abundance of carbonyl compounds and the total carbonyl compound concentrations in the different water types

3.4.1.1. Relative abundance of the quantified carbonyl compounds. After laboratory-scale ozonation, 8 out of the 17 target carbonyl compounds were detected above the LOQs in different water types in laboratory-scale experiments (Figs. 4 and 5). Four carbonyl compounds (benzaldehyde, cyclopentanone, decanal, and 3,5-heptanedione) were only detected < LOQs. Fig. 4 shows the sum of the molar concentrations of carbonyl compounds (pie chart size) and the fractional molar distribution of the 8 target carbonyl compounds for ozonated samples, for a specific ozone dose of 0.5 mgO_3/mgC (for mass concentrations see Figure S9 (SI)). The relative molar abundance of the target carbonyl compounds in most samples decreased in the following order: formaldehyde > acetaldehyde > glyoxylic acid > pyruvic acid > glutaraldehyde > 2,3-butanedione > glyoxal > 1-acetyl-1-cyclohexene. Formaldehyde and acetaldehyde accounted for > 80% of the total molar carbonyl concentration in most wastewater samples, in line with the findings of Marron et al. (2020). Formaldehyde was also detected in all water types before ozonation except Werdhölzli October 2021 wastewater and Lac de Bret (Fig. 5). Other carbonyl compounds such as acetaldehyde and 1-acetyl-1-cyclohexene were also detected > LOQs in some non-ozonated samples (Fig. 5). The occurrence of formaldehyde and acetaldehyde in non-ozonated lake water and secondary wastewater effluent has been reported previously (Glaze et al., 1989; Marron et al., 2020; Wert et al., 2007).

In the two lake waters, the fractional molar distribution differed significantly from one another, with the most abundant carbonyl compounds being formaldehyde (92%) and acetaldehyde (96%) in Lake Greifensee and Lac de Bret, respectively. The lower number of carbonyl compounds quantified in ozonated lake waters (DOC 3.1–3.4 mg/L) compared to wastewaters (DOC 4.7–10.9 mg/L) is attributed to the lower DOC concentrations and/or different DOM type.

3.4.1.2. Total carbonyl compound concentration for the different water types. Higher total carbonyl compounds concentrations were measured in ozonated wastewater and SRFA-containing water compared to lake water (pie chart size, Fig. 4). To assess the role of the DOM type, the concentrations of carbonyl compounds were normalised to the DOC (Figure S12, SI). At a specific ozone dose of 0.5 mgO_3/mgC , the DOC-normalised total carbonyl compounds concentrations were 0.1 and 0.8 $\mu M/mgC$ in Lac de Bret and Lake Greifensee, respectively, ranged within 0.5 – 1.1 $\mu M/mgC$ in wastewater, and reached 1.5 $\mu M/mgC$ for SRFA. In Werdhölzli wastewater, the DOC concentration-normalised total carbonyl compounds concentrations detected in September 2020 and March 2021 samples were almost identical (around 1 $\mu M/mgC$), while in October 2021 samples it was significantly lower (0.5 $\mu M/mgC$). The lower concentration of formaldehyde in the October 2021 samples explains most of this difference (Figure S12, SI). At a specific ozone dose of ca. 2 mgO_3/mgC , the DOC concentration-normalised total carbonyl compounds concentrations ranged from 0.03 – 0.5 $\mu M/mgC$ in lake waters, 0.9 – 1.6 $\mu M/mgC$ in wastewaters, and reached 1.9 $\mu M/mgC$ in SRFA-containing waters (Figure S12, SI). This is in agreement with the

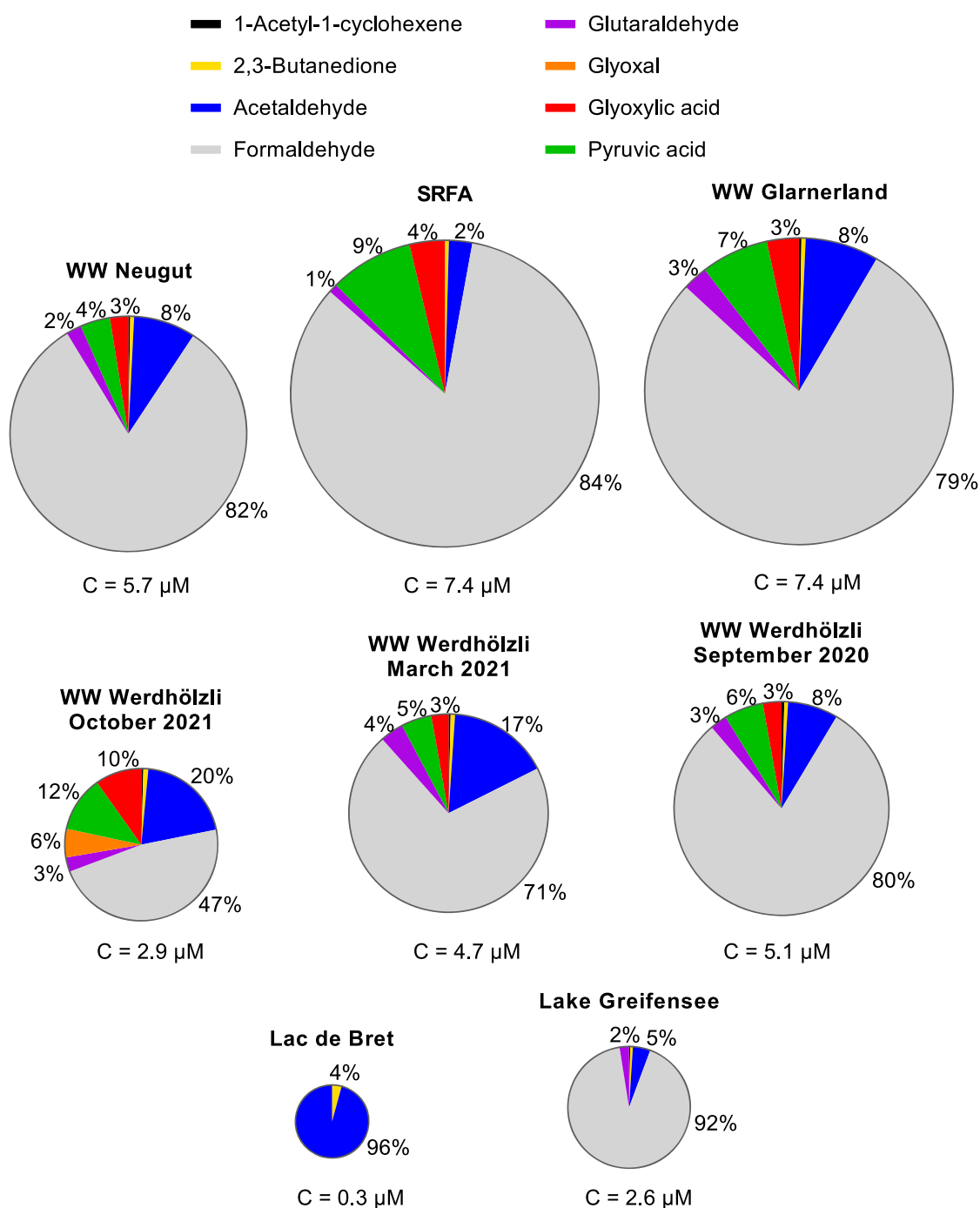


Fig. 4. Fractional distribution of the 8 quantified target carbonyl compounds based on molar concentrations in the ozonated water and wastewater (WW) samples at a specific ozone dose of 0.5 mgO₃/mgC. Percentage values correspond to the molar concentrations of individual target carbonyl compounds relative to the sum of 8 target carbonyl compounds detected in the ozonated sample. The size of the pie charts is proportional to the sum of the molar carbonyl compounds concentrations in the different samples, which is provided as C in μM.

broad formation range reported previously during drinking water ozonation where aldehyde formation ranged within 0.1 and 0.5 μM/mgC (of TOC) (Weinberg et al., 1993). Furthermore, significant differences in carbonyl compound formation in different lake waters have been reported at similar pH, total organic carbon (TOC) and ozone doses (Schechter 1993; Weinberg and Glaze 1996; Weinberg et al., 1993).

These findings highlight the role of the DOM type in the formation of carbonyl compounds during ozonation. The high formation potential of

carbonyl compounds in SRFA-containing waters and secondary wastewater effluent DOM is in line with recent findings of Houska et al. (2021) who measured high phenol concentrations in SRFA (2.84 μM phenol per gC) and in effluent DOM isolate and wastewaters (1.69–2.17 μM phenol per gC), while the concentrations of phenolic moieties were not measured in lake waters. Additionally, Houska et al. (2023) reported a relationship between carbonyl compound formation and the electron donating capacity (EDC), which further supports this hypothesis.

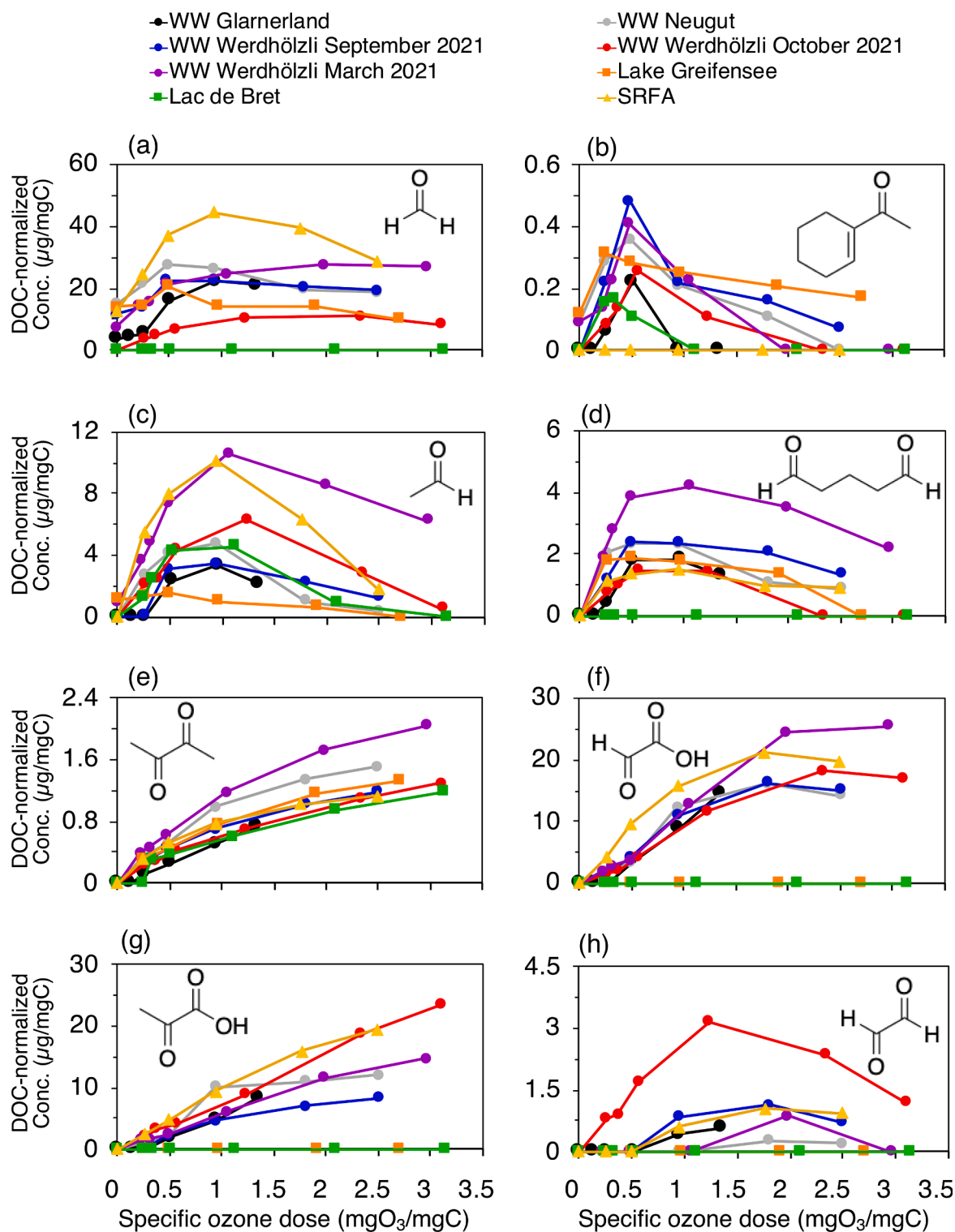


Fig. 5. DOC concentration-normalised concentrations of 8 target carbonyl compounds as a function of the specific ozone doses in the different water types (see legend); (a) formaldehyde, (b) 1-acetyl-1-cyclohexene, (c) acetaldehyde, (d) glutaraldehyde, (e) 2,3-butanedione, (f) glyoxylic acid, (g) pyruvic acid, and (h) glyoxal. The structure of each compound is shown in the corresponding panel.

3.4.2. Formation trends of the target carbonyl compounds in the different water types

Based on the variation of the concentrations of carbonyl compounds as a function of the specific ozone doses (laboratory-scale ozonation) applied in the present study, multiple formation trends could be distinguished for the quantified target carbonyl compounds (Fig. 5). The same formation trends were observed for the non-target carbonyl compounds and are discussed in detail with mechanistic considerations in Houska et al. (2023). In brief, based on its formation trend, a carbonyl compound can be classified into one of the following five categories:

- (i) Primary product with constant maximum (formaldehyde, Fig. 5a)
- (ii) Primary product with further abatement (1-acetyl-1-cyclohexene, acetaldehyde, glutaraldehyde, Figs. 5b, c, d)
- (iii) Product with direct but less efficient formation (2,3-butanedione, Fig. 5e)
- (iv) Secondary product with constant maximum (glyoxylic acid, pyruvic acid and potentially glyoxal, Figs. 5f, g, h)
- (v) Secondary product with further abatement (only non-target compounds, Houska et al., 2023)

The variability in the formation trends of carbonyl compounds can be explained by differences in their formation kinetics/mechanisms from precursors, precursor concentrations and abatement efficiencies, which are governed by their reactivities with ozone and $\cdot\text{OH}$ (Buxton et al., 1988; Hoigné and Bader 1983a; Houska et al. 2023; von Sonntag and von Gunten 2012).

The pH did not influence the formation of carbonyl compounds significantly, which was demonstrated by similar concentrations of carbonyl compounds for samples with and without pH adjustment (pH 7, Werdhölzli September 2021, Glarnerland, and Neugut versus pH \sim 8, Werdhölzli October and March 2021). This is consistent with the findings of a previous study in which only a minor decrease in aldehyde concentration was reported as the pH increased from 7 to 8 (Hoigné 1998).

- (a) **Formaldehyde** (Fig. 5a, trend (i), LOQ = 15.01 $\mu\text{g/L}$) concentrations before ozonation varied from $< \text{LOQ}$ to 74.72 $\mu\text{g/L}$ (Figure S10a, SI). The formation trend of formaldehyde consisted of increasing concentrations during ozonation until a peak at either 0.5 mgO_3/mgC (WW Werdhölzli September 2021, Lake Greifensee water, and WW Neugut), 1 mgO_3/mgC (WW Glarnerland and SRFA), or 2–3 mgO_3/mgC (WW Werdhölzli, March and October 2021). Beyond this point, formaldehyde concentrations levelled off or decreased slightly, in agreement with the trends reported previously (Can and Gurol 2003; Huang et al., 2005). Such a trend can be explained by an expected continuous formation of formaldehyde from precursors (which are not fully depleted) during ozonation along with low reactivities of

formaldehyde with ozone ($k = 0.1 \text{ M}^{-1}\text{s}^{-1}$) (Hoigné and Bader 1983a) and $\cdot\text{OH}$ ($k = 1.0 \cdot 10^9 \text{ M}^{-1}\text{s}^{-1}$) (Buxton et al., 1988). The highest DOC concentration-normalised formaldehyde formation was observed during ozonation of SRFA (44.5 $\mu\text{g}/\text{mgC}$), whereas it varied considerably in WW in the range of 10.9–27.6 $\mu\text{g}/\text{mgC}$ and in the two lake water samples ($< \text{LOQ}$ or 20.8 $\mu\text{g}/\text{mgC}$, in Lac de Bret or Lake Greifensee, respectively). In comparison to previous studies, formaldehyde concentrations in wastewater were similar or up to two-fold higher (Marron et al., 2020; Wert et al., 2007), while in lake waters they were similar to previous studies (Huang et al., 2005; Marron et al., 2020) (Fig. 6).

- (b) **1-Acetyl-1-cyclohexene** (Fig. 5b, trend (ii), LOQ = 0.37 $\mu\text{g/L}$) was detected in non-ozonated secondary wastewater effluent $< \text{LOQ}$ – 0.46 $\mu\text{g/L}$ and in Lake Greifensee water at 0.41 $\mu\text{g/L}$ (Figure S10b, SI). Its concentration increased during ozonation up to a specific ozone dose of 0.3 mgO_3/mgC in lake water and 0.5 mgO_3/mgC in wastewater, above which it decreased (Fig. 5b). The abatement of 1-acetyl-1-cyclohexene at high ozone doses can be attributed to its further ozone reaction at the olefin group with $k_{\text{O}_3} = 2 \cdot 10^5 \text{ M}^{-1}\text{s}^{-1}$ at 20 $^\circ\text{C}$ (Wang et al. in preparation). Due to this reactivity, the ozone reaction is expected to dominate the abatement of 1-acetyl-1-cyclohexene even though it has an expected high second-order rate constant for the reaction with $\cdot\text{OH}$, similar to cyclohexene ($k_{\text{OH}} = 8.8 \cdot 10^9 \text{ M}^{-1}\text{s}^{-1}$) (Michael and Hart 1970). The formation of 1-acetyl-1-cyclohexene in wastewater was slightly higher (0.3 – 0.5 $\mu\text{g}/\text{mgC}$) than in lake water (0.2 – 0.3 $\mu\text{g}/\text{mgC}$), while in SRFA it was negligible ($< \text{LOQ}$) (Fig. 5b).
- (c) **Acetaldehyde** (Fig. 5c, trend (ii), LOQ = 1.32 $\mu\text{g/L}$) was detected in secondary wastewater effluent at concentrations $< \text{LOQ}$ to 4.45 $\mu\text{g/L}$ (Figure S10c, SI). Acetaldehyde increased during ozonation up to a specific ozone dose of 1 mgO_3/mgC followed by a decrease (Fig. 5c). This decrease occurring only at high specific ozone doses can be explained by a limited reactivity with ozone ($k = 1.5 \text{ M}^{-1}\text{s}^{-1}$) (Hoigné and Bader 1983a) but a significant reactivity with $\cdot\text{OH}$ ($k = 2.4 \cdot 10^9 \text{ M}^{-1}\text{s}^{-1}$) (Schuchmann and von Sonntag 1988). The formation of acetaldehyde varied significantly between water types and even amongst the different samples of the same water type. The DOC concentration-normalised maximum formation varied between 3.4 and 10.6 $\mu\text{g}/\text{mgC}$ in wastewater. In SRFA, the DOC concentration-normalised maximum formation (10.1 $\mu\text{g}/\text{mgC}$) was similar to wastewater, while in lake waters it was significantly lower (1.5 – 4.6 $\mu\text{g}/\text{mgC}$). The concentrations measured in wastewater are within the range of concentrations reported previously in similar water types (Fig. 6) (Marron et al., 2020; Wert et al., 2007), whereas in lake water they are slightly lower than in previous studies (Huang et al., 2005; Marron et al., 2020). However, considering the three-fold difference between the two

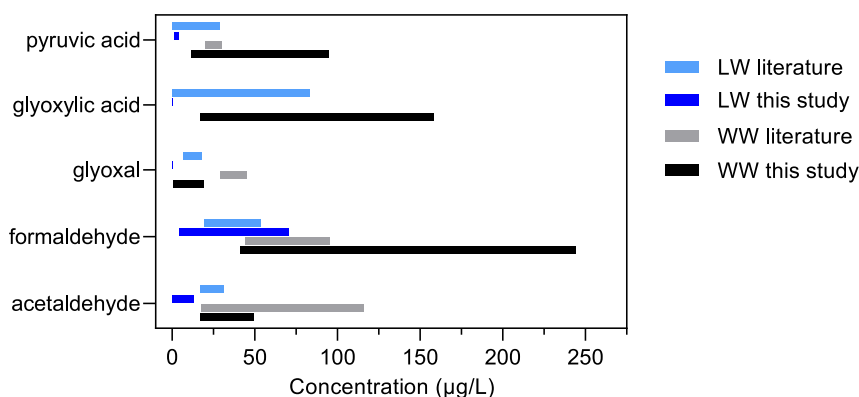


Fig. 6. Comparison of concentration ranges for selected carbonyl compounds formed during ozonation of lakewater (LW) and secondary wastewater effluent (WW) in the current and previous studies at specific ozone doses of 0.4–2 mgO_3/mgC (Hammes et al., 2006; Huang et al., 2005; Marron et al., 2020; Wert et al., 2007).

lake water types in the present study, the observed differences to previous studies can be expected.

- (d) *Glutaraldehyde* (Fig. 5d, trend (ii), LOQ = 4.51 µg/L) was < LOQ in all samples before ozonation. During ozonation, glutaraldehyde concentrations increased up to specific ozone doses of 0.5–1 mgO₃/mgC, followed by a decrease (Fig. 5d). Low ozone reactivity is expected for this aliphatic compound, whereas $\bullet\text{OH}$ can lead to a slow abatement with an estimated $k \sim 2\text{--}3 \cdot 10^9 \text{ M}^{-1}\text{s}^{-1}$ based on the alkane (propane and butane) reactivity (Getoff 1991). The DOC concentration-normalised glutaraldehyde formation was generally higher in wastewater (1.4 – 4.2 µg/mgC) than in lake water (< LOQ – 1.9 µg/mgC) and SRFA (1.5 µg/mgC) (Fig. 5d). Glutaraldehyde has not been reported in ozonated lake water or wastewater previously.
- (e) *2,3-Butanedione* (Fig. 5e, trend (iii), LOQ = 0.86 µg/L) was < LOQ in all water and wastewater samples before ozonation. 2,3-butanedione is the only carbonyl compound without a carboxylic acid functional group that increased continuously with increasing ozone doses (Fig. 5e). The concentration increase is direct and steady but not steep and thus it is classified as a compound with trend (iii). The continuous increase of 2,3-butanedione can be explained by a presumed slow continuous formation from residual precursors during ozonation and an expected low reactivity with ozone (expected $k < 1 \text{ M}^{-1}\text{s}^{-1}$) and $\bullet\text{OH}$ ($k = 1.7 \cdot 10^8 \text{ M}^{-1}\text{s}^{-1}$) (Buxton et al., 1988). The maximum formation of 2, 3-butanedione ranged between 1.2 and 2.0 µg/mgC in the different water types, with the highest formation in wastewater samples reaching 2.0 µg/mgC at 3 mgO₃/mgC (Fig. 5e). A molecular formula suspected as 2,3-butanedione has been detected in a previous study upon ozonation of drinking water (Richardson et al., 1999), however, it was not quantified.
- (f) *Glyoxylic acid* (Fig. 5f, trend (iv), LOQ = 3.33 µg/L) was either not detected or < LOQ in all samples before ozonation. Glyoxylic acid concentrations increased with mostly a lag-phase but steadily upon ozonation reaching a plateau at a specific ozone dose of about 2 mgO₃/mgC (Fig. 5f). The DOC concentration-normalised formation of glyoxylic acid ranged between 16.2 and 25.4 µg/mgC in wastewater, while it reached 21.2 µg/mgC in SRFA-containing solutions. In lake waters, glyoxylic acid concentrations remained < LOQ after ozonation. Previously, a wide range of glyoxylic acid concentrations has been reported (Fig. 6). Huang et al. (2005) reported high glyoxylic acid formation ($\leq \sim 130 \text{ µg/L}$) during ozonation of lake water at 3 mgO₃/mgC, which is comparable to the maximum concentration measured in SRFA-containing solutions (106 µg/L) at 1.76 mgO₃/mgC in this study. Hammes et al. (2006) reported negligible and low glyoxylic acid concentrations (< LOQ and 2.5 µg/L) in ozonated samples from Lake Greifensee and Lake Zurich, respectively. The trend of glyoxylic acid can be explained by its low reactivity with ozone, with $k = 1.9 \text{ M}^{-1}\text{s}^{-1}$ (for the deprotonated species glyoxylate) (Hoigné and Bader 1983b), and an expected low reactivity with $\bullet\text{OH}$.
- (g) *Pyruvic acid* (Fig. 5g, trend (iv), LOQ = 3.96 µg/L) was not detected or < LOQ in wastewater samples before ozonation (Figure S10g, SI). Unlike glyoxylic acid, for which the concentrations tended to level off or slightly decrease at very high ozone doses, pyruvic acid concentrations continuously increased with increasing ozone doses (Fig. 5g). The DOC concentration-normalised concentrations of pyruvic acid varied amongst the wastewater samples in the range of 8.3 and 23.5 µg/mgC, reached 19.5 µg/mgC in SRFA-containing water, and remained < LOQ in lake water samples (Fig. 5g). The concentrations of pyruvic acid measured in ozonated wastewater in the current study (45.6–140.9 µg/L) are higher than those reported by Wert et al. (2007), where the pyruvic acid concentration reached 41 µg/L in tertiary wastewater effluent and decreased during ozonation

(Fig. 6). The trend reported by Wert et al. (2007) is inconsistent with the trend observed in the current study and other studies where the formation of pyruvic acid increased during ozonation (Huang et al., 2005).

- (h) *Glyoxal* (Fig. 5h, potentially trend (iv), LOQ = 0.58 µg/L) was highly unstable after derivatisation (disappeared completely after 1 day, see stability assessment in Section 3.2), while in non-derivatised samples, its concentration increased during storage, which may result from the decay of organic peroxo compounds formed during Criegee-type reactions (von Sonntag and von Gunten 2012; Wang et al., prep.). Due to its instability, a full quantitative assessment of glyoxal formation in the different water types and an unequivocal trend assignment were not possible. Before ozonation, glyoxal was not detected in any of the samples. Glyoxal concentrations increased slightly at low ozone doses and peaked at specific ozone doses of 1 – 2 mgO₃/mgC, followed by a decrease. The latter is likely due to the instability of derivatised glyoxal rather than due to glyoxal abatement, given its expected low reactivity with ozone (expected $k < 0.1 \text{ M}^{-1}\text{s}^{-1}$) and $\bullet\text{OH}$ ($k = 6.6 \cdot 10^7 \text{ M}^{-1}\text{s}^{-1}$) (Buxton et al., 1988). Therefore, the glyoxal formation trend is tentatively assigned as trend (iv). The DOC concentration-normalised formation of glyoxal ranged from 0.3 to 3.2 µg/mgC in wastewater, was 1.0 µg/mgC in SRFA-containing water, while it was negligible (< LOQ) in lake waters (Fig. 5h). Glyoxal concentrations measured in ozonated samples in the current study (<LOQ – 19.0 µg/L) are lower than concentrations reported previously (7 – 71 µg/L) (Fig. 6). This disparity might be caused by the higher instability of the TSH-derivatised glyoxal compared to the other derivatisation methods or different storage times in previous studies before or after derivatisation.

3.5. Formation and fate of carbonyl compounds in full-scale WWTPs

3.5.1. Comparison of the formation of carbonyl compounds during laboratory and full-scale ozonation

Fig. 7 shows a comparison of the formation of carbonyl compounds during laboratory- and full-scale ozonation (based on WW Werdhölzli March 2021 samples). The concentrations of 1-acetyl-1-cyclohexene, 2,3-butanedione, formaldehyde, and glutaraldehyde were in a similar range for similar specific ozone doses, whereas for acetaldehyde, glyoxylic acid, and pyruvic acid, the concentrations were considerably different (Fig. 7). The acetaldehyde concentrations were more than two-fold higher in laboratory- than in the plant-ozonated samples. On the contrary, glyoxylic acid and pyruvic acid concentrations were higher in full-scale ozonation, except at the lowest ozone dose (0.3 mgO₃/mgC) where they were only detected in laboratory experiments (Fig. 7).

Despite this discrepancy for some compounds, the total target carbonyl concentrations were generally in a similar range ($\sim 20\%$ difference) for the laboratory- and the full-scale ozonation at moderate and high specific ozone doses considering the slightly different specific ozone doses (Fig. 7). However, the difference was more pronounced (up to 40% in total concentration) at a low ozone dose, which may be attributed to a more significant influence of pH and temperature variability on the ozone exposure and reactivity in comparison to at high ozone doses where such effects become less important due to the higher ozone exposures (von Sonntag and von Gunten 2012).

One main difference between laboratory- and full-scale ozonation was a pre-filtration (at 0.45 µm) before laboratory ozonation. However, the formation of target and non-target carbonyl compounds during laboratory ozonation experiments in both filtered and non-filtered secondary wastewater effluent was quite similar (Figure S13, SI). Therefore, an influence of particulate organic matter as a cause of the observed disparity can be ruled out.

Similar to the trends observed in laboratory ozonation, the concentrations of carbonyl compounds generally increased at the applied

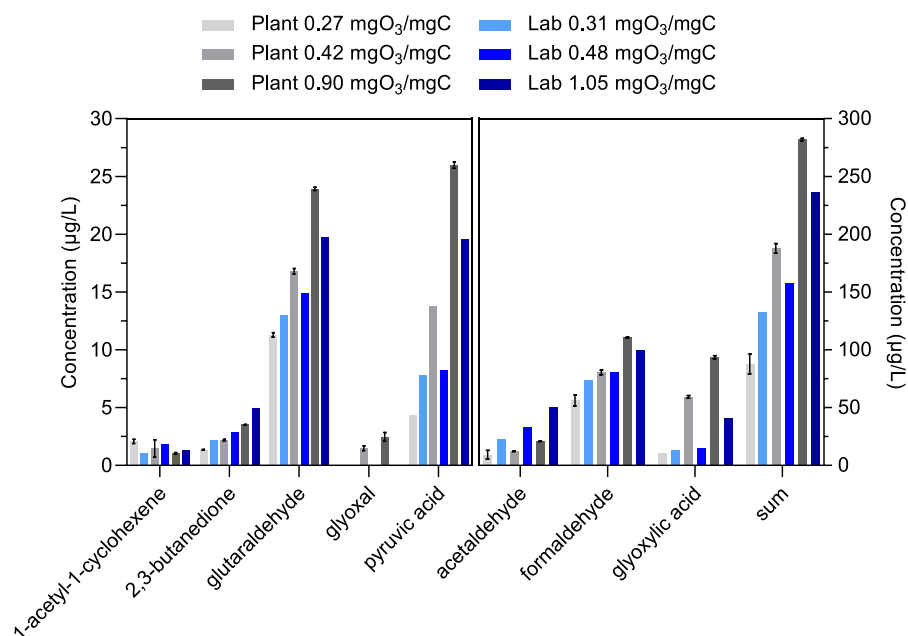


Fig. 7. Concentrations of selected carbonyl compounds and their sum in laboratory- and full-scale ozonation (same secondary wastewater effluent) for various specific ozone doses. Error bars correspond to the range based on duplicate samples (only available for plant-ozonated samples). Experimental conditions: for laboratory ozonation, pH = 7.80–7.94, $T = 22\text{ }^{\circ}\text{C}$; for full-scale ozonation (in the ozone reactor at the plant) pH = 6.98–6.99, $T = 15.4\text{--}15.7\text{ }^{\circ}\text{C}$. Concentrations of acetaldehyde, formaldehyde, glyoxylic acid and the total carbonyl compounds are displayed on the secondary y-axis.

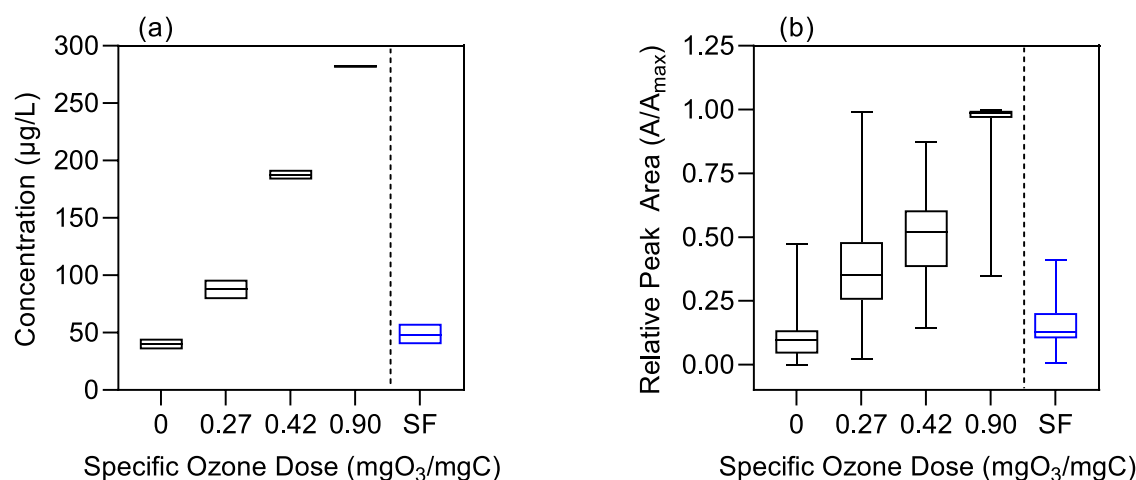


Fig. 8. Fate of (a) target and (b) non-target carbonyl compounds during full-scale wastewater ozonation (Werdhölzli) as a function of the specific ozone dose and after biological sand filtration (SF) ($n = 6, 2, 2, 2$, and 6 for specific ozone dose 0, 0.27, 0.42, 0.90 mgO_3/mgC , and SF effluent samples for all specific ozone doses, respectively). (a) Total concentration of the 8 target carbonyl compounds. Boxes show the concentration range and the lines inside correspond to the mean concentration. (b) Relative peak areas (A/A_{max} , with A = area in each sample type and A_{max} = highest area measured in all samples) of non-target carbonyl compounds ($n = 40$) for which peak areas in the full-scale wastewater samples were determined. Lines inside the boxes correspond to the first quartile and the whiskers correspond to the minimum and maximum concentrations (including outliers).

specific ozone dose range of 0.3–1 mgO_3/mgC in the full-scale system (Figs. 7 and 8). The maximum total concentrations of target carbonyl compounds were 237 and 282 $\mu\text{g/L}$ for laboratory- and full-scale ozonated wastewaters, respectively.

3.5.2. Fate of carbonyl compounds during biological sand filtration

During biological sand filtration, the concentrations of all target carbonyl compounds were abated significantly, with an overall abatement of the total carbonyl compound concentrations of 57–86%, depending on the specific ozone dose and hence the carbonyl compound concentration (Fig. 8a). The abatement of individual target carbonyl compounds (in ozonated wastewater at 0.9 mgO_3/mgC) was > 64–94% (Figure S14, SI). For compounds abated to levels below LOQ, the latter was used as the final concentration to calculate the % abatement, which is therefore a minimum. The only target carbonyl compounds detected after biological sand filtration were in decreasing

concentration order (Figure S14, SI): formaldehyde (in all samples), glyoxylic acid (in 66% of samples), glutaraldehyde (in 33% samples), and 1-acetyl-1-cyclohexene (in 66% of samples) (Figure S14, SI). The bulk of the total carbonyl compound concentration after biological sand filtration was caused by formaldehyde, which had concentrations in a similar range as in the secondary wastewater effluent before ozonation (Figure S14, SI).

Similarly, non-target carbonyl compounds were mostly abated significantly during biological sand filtration, with an average abatement of 84% (by comparing the peak area of non-target carbonyl compounds in sand filtration effluent to the peak area in ozonated wastewater at 0.9 mgO_3/mgC), approaching the peak area measured in secondary wastewater effluent before ozonation (Fig. 8b). For the non-target carbonyl compounds, the abatement of individual compounds ranged between 47 and 100%. Excluding the peak area of a non-target carbonyl compound initially occurring in secondary wastewater

effluent, the abatement of the fraction formed during ozonation (at three ozone doses) was > 88% for all individual carbonyl compounds. The overall abatement ranged between 109 and 120% for all ozonated wastewater samples. However, these abatement percentages for the non-target carbonyl compounds only represent a tentative estimate, due to the uncertainty arising from the use of peak areas instead of concentrations.

The abatement of carbonyl compounds reported here is in agreement with results from previous studies in which selected target carbonyl compounds were degradable during biological filtration processes (sand and granular activated carbon filtration) in both lake water and wastewater (Carlson and Amy 1998; Digiano et al., 2001; Kramer et al., 1993; Levine et al., 2000; Marron et al., 2020; Weinberg and Glaze 1996; Weinberg et al., 1993). In previous studies, the abatement of carbonyl compounds was shown for only a selection of carbonyl compounds, while in this study the biodegradability is demonstrated for 48 carbonyl compounds (targets and non-targets) and confirmed as a characteristic for this class of compounds. Moreover, the trend of carbonyl compounds formation during ozonation followed by an abatement during biological filtration is comparable to previously reported trends for AOC in lake water and wastewater (Hammes et al., 2006; Zimmermann et al., 2011). AOC has been shown to increase significantly during ozonation of lake water and secondary wastewater followed by a partial or complete abatement during sand filtration leading to AOC concentrations in a similar range to those before ozonation. This trend similarity is consistent with the known role of carbonyl compounds as a main fraction of AOC in ozonated water and wastewater (von Sonntag and von Gunten 2012).

The formation of carbonyl compounds during ozonation and their subsequent abatement during sand filtration is in line with previously reported toxicity evolution trends consisting of an increase of toxicity (mainly mutagenicity) during ozonation followed by a decrease during biological treatment (Kienle et al., 2022; Magdeburg et al., 2014; Stalter et al., 2010; Volker et al., 2019). This hints towards a role of carbonyl compounds as a contributor to the detected toxic effects in ozonated wastewater, in line with previous hypotheses regarding the susceptibility of oxygen-rich DBPs to induce toxicity in treated water (von Gunten 2018). The ability of many aldehydes, including some of the compounds formed during ozonation (e.g., formaldehyde, acetaldehyde, glyoxal, glutaraldehyde) and others to induce DNA mutations or other forms of genotoxicity, has been previously demonstrated (Antonowicz et al., 2021; Demkowicz-Dobrzanski and Castonguay 1991; Grafstrom et al., 1983; Mukai and Goldstein 1976; NIOSH Current Intelligence Bulletin 55 1991; Weng et al., 2018).

4. Conclusions

- TSH derivatisation coupled to an advanced HRMS-based non-target workflow provides a simple and robust approach for the targeted and non-targeted analysis of carbonyl compounds in (non-)ozonated surface water and wastewater.
- Evaluation of different derivatisation conditions, the stability of (derivatised) carbonyl compounds, and the effects of sample filtration on the formation of carbonyl compounds during ozonation revealed best practices for sample preparation.
- Ozonation of lake water, SRFA-containing water, and secondary wastewater effluent resulted in the formation of carbonyl compounds. 8 out of 17 target carbonyl compounds were detected > LOQs in most ozonated samples.
- The DOM type played a predominant role in the formation of carbonyl compounds, since the DOC concentration-normalised carbonyl compound concentrations were higher in SRFA (1.9 µM/mgC) and wastewater (0.9–1.6 µM/mgC) compared to lake water (0.03–0.5 µM/mgC) at a specific ozone dose of 2 mgO₃/mgC.
- The abundance of carbonyl compounds differed significantly between the two ozonated lake waters, while in ozonated wastewater

and SRFA-containing water it was comparable. The molar concentrations decreased in the order: formaldehyde > acetaldehyde > glyoxylic acid > pyruvic acid > glutaraldehyde > 2,3-butanedione > glyoxal > 1-acetyl-1-cyclohexene.

- Formation trends were assigned to the different quantified target carbonyl compounds. They depend on the reactivities and concentrations of precursors, the reactivity of target compounds with ozone and hydroxyl radical, and the stability of these two oxidants.
- The formation of carbonyl compounds in laboratory- and full-scale ozonation of wastewater was similar for all target compounds except acetaldehyde, glyoxylic acid, and pyruvic acid. The total carbonyl concentrations diverged by around 20% at moderate and high specific ozone doses.
- The concentrations and peak intensities of target and non-target carbonyl compounds, respectively, decreased significantly during biological sand filtration at a full-scale wastewater treatment plant. This highlights the biodegradability of this class of compounds and the importance of biological post-treatment after ozonation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2023.119751.

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