

Ozonation of lake water and wastewater: Identification of carbonous and nitrogenous carbonyl-containing oxidation byproducts by non-target screening

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ABSTRACT

Ozonation of drinking water and wastewater is accompanied by the formation of disinfection byproducts (DBPs) such as low molecular weight aldehydes and ketones from the reactions of ozone with dissolved organic matter (DOM). By applying a recently developed non-target workflow, 178 carbonous and nitrogenous carbonyl compounds were detected during bench-scale ozonation of two lake waters and three secondary wastewater effluent samples and full-scale ozonation of secondary treated wastewater effluent. An overlapping subset of carbonyl compounds (20%) was detected in all water types. Moreover, wastewater effluents showed a significantly higher fraction of *N*-containing carbonyl compounds (30%) compared to lake water (17%). All carbonyl compounds can be classified in 5 main formation trends as a function of increasing specific ozone doses. Formation trends upon ozonation and comparison of results in presence and absence of the \bullet OH radical scavenger DMSO in combination with kinetic and mechanistic information allowed to elucidate potential carbonyl structures. A link between the detected carbonyl compounds and their precursors was established by ozonating six model compounds (phenol, 4-ethylphenol, 4-methoxyphenol, sorbic acid, 3-buten-2-ol and acetylacetone). About one third of the detected carbonous carbonyl compounds detected in real waters was also detected by ozonating model compounds.

Evaluation of the non-target analysis data revealed the identity of 15 carbonyl compounds, including hydroxylated aldehydes and ketones (e.g. hydroxyacetone, confidence level (CL) = 1), unsaturated dicarbonyls (e.g. acrolein, CL = 1; 2-buten-1,4-dial, CL = 1; 4-oxobut-2-enoic acid, CL = 2) and also a nitrogen-containing carbonyl compound (2-oxo-propanamide, CL = 1).

Overall, this study shows the formation of versatile carbonous and nitrogenous carbonyl compounds upon ozonation involving ozone and \bullet OH reactions. Carbonyl compounds with unknown toxicity might be formed, and it could be demonstrated that acrolein, malondialdehyde, methyl glyoxal, 2-buten-1,4-dial and 4-oxo-pentenal are degraded during biological post-treatment.

1. Introduction

Chemical disinfection and oxidation of water and wastewater is typically accompanied by reactions with water matrix components such as dissolved organic matter (DOM), which leads to undesired disinfection or oxidation byproducts (DBPs or OBPs) (Bond et al., 2011; Li and Mitch, 2018; Richardson, 2011; von Gunten, 2018).

To date, over 700 DBPs have been identified from various chemical oxidants (Richardson and Kimura, 2019). Water stress increases the use

of impaired water resources, which aggravates the problem of undesired reactions of the applied oxidants with water matrix components, because of higher dissolved organic carbon (DOC) concentrations and a more heterogeneous DOM composition. Chemical oxidants can lead to a formation of carbonyl-containing DBPs, with ozone having the most extensive formation of this compound class (Ramseier et al., 2011; Richardson, 2011; Richardson et al., 1999; von Gunten, 2018; von Sonntag and von Gunten, 2012). Carbonyl compounds have gained increasing attention due to their potential toxicity (Lopachin and Gavin,

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2014; Prasse et al., 2018; Tentscher et al., 2018). During ozonation, oxidation of DOM occurs by ozone and/or hydroxyl radicals ($\bullet\text{OH}$), which are formed from ozone decomposition (Buffle and von Gunten, 2006; von Sonntag and von Gunten, 2012). Ozone is a relatively selective oxidant reacting mainly with electron-rich moieties such as olefins, activated aromatic systems and neutral amines, whereas $\bullet\text{OH}$ are less selective with most reactions close to diffusion control (Buffle and von Gunten, 2006; Lim et al., 2022; Staehelin and Hoigne, 1985; von Gunten, 2003). One prominent ozone reaction type is the Criegee mechanism, which leads to a scission of a C-C double bond with the ensuing carbonyl formation (Lim et al., 2022; von Sonntag and von Gunten, 2012). Reactions with $\bullet\text{OH}$ typically lead to C-centred radicals which, in the presence of dissolved oxygen, further react to peroxy radicals and finally decay to alcohols, ketones and aldehydes (Kamath et al., 2018; von Sonntag et al., 1997). Thus, both reaction pathways may lead to carbonyl compounds such as carboxylic acids, aldehydes and ketones which typically are easily biodegradable and thus contribute to the biodegradable and/or assimilable organic carbon (BDOC/AOC). However, also potentially toxic *p*-benzoquinones, catechols or α,β -unsaturated carbonyl compounds may be formed (Glaze, 1986; Hammes et al., 2006; Marron et al., 2020; Nawrocki et al., 2003; Prasse et al., 2018; Richardson et al., 1999; Tentscher et al., 2018). The aforementioned compounds might be formed from phenolic compounds which are predominant precursors in DOM (Houska et al., 2021; Önnby et al., 2018; Rougé et al., 2020a, 2020b; Walpen et al., 2020). Carbonyl compounds are also potential precursors of halogenated disinfection byproducts, which are formed during reactions with free or combined chlorine (i.e. chloramines) and may even lead to an increase in toxicity or number of DBPs in distribution systems (Marron et al., 2021; Phungsai et al., 2019). In many cases, ozonation but also chlorination is followed by a biological post-treatment, which in general leads to a reduction of AOC, toxicity and the number of DBPs observed in the finished water (Gulde et al., 2021; Hammes et al., 2006; Krasner, 2009; Philip Singer, 1994; Shi et al., 2021; van der Kooij et al., 1989; Völker et al., 2019).

High-resolution mass spectrometry can be applied to investigate the formation of ozonation-induced byproducts with molecular weights > 100–150 (Jennings et al., 2022; Phungsai et al., 2016; Remucal et al., 2020; These and Reemtsma, 2005; Zhang et al., 2021). Complementary, chemical derivatisation procedures have been applied to enable the analysis of compounds with challenging physicochemical properties such as carbonyl compounds (e.g. hydrophilic, low molecular weight or poorly ionisable) (Siegel et al., 2014; Vogel et al., 2000). Recently, cross-linking reactions with common nucleophiles enabled the identification of certain electrophilic α,β -unsaturated carbonyl compounds (Prasse et al., 2018). With these approaches so far about 50 carbonous (di)aldehydes, (di)ketones and ketoacids have been proposed to be formed during ozonation with only a part of them unequivocally confirmed or quantified (Hammes et al., 2006; Liu et al., 2015; Marron et al., 2020; Richardson et al., 1999; Świetlik et al., 2004; Tentscher et al., 2018; Weinberg, 1999; Wert et al., 2007).

Besides carbonous, also nitrogenous byproducts may be formed. The most prominent example is the carcinogenic *N*-nitrosodimethylamine (NDMA) which is e.g. formed from ozonation of precursors such as *N*,*N*-dimethylsulfamide (DMS) or dimethylamine (DMA) (Andrzejewski et al., 2008; Schmidt and Brauch, 2008; von Gunten et al., 2010; Zhao et al., 2008). Further, nitromethane is a ubiquitous wastewater ozonation byproduct (Shi et al., 2021). Water matrix components such as dissolved organic nitrogen (DON) or nitrite are potential precursors of N-DBPs (Thibaud et al., 1987; Westerhoff and Mash, 2002). DON components such as amides, pyrroles, purines and pyrimidines do not release high yields of nitrate upon ozonation, suggesting that their N atom might appear in ozonation-induced byproducts (Essaïed et al., 2022). Despite the identification of many different N-DBPs, the formation of nitrogenous carbonyl compounds has not been shown yet.

To date, no systematic investigation has been performed to identify unknown carbonyl compounds formed during ozonation of real water samples. Only the study by Liu et al., 2020 elucidated carbonyl compounds derived from oxidised SRFA. Their strategy involved stable isotope labelling after sample preconcentration and demonstrated the formation of unknown carbonous carbonyl compounds in oxidised DOM isolates with discussion of potential DOM precursors (Liu et al., 2020). The use of *p*-toluenesulfonylhydrazine as derivatising agent without any preconcentration was applied to measure selected carbonyl compounds in biological samples (Siegel et al., 2014) or water reuse systems (Marron et al., 2020). In a companion study, this method was enhanced and systematically validated and now allows tracking of signature fragments from chromatographic separated signals for the identification of unknown, non-targeted carbonyl compounds (Manasfi et al., 2023).

The aims of this study are (1) to evaluate the formation of carbonyl compounds at varying specific ozone doses in lake waters and wastewaters, (2) to explore a link between carbonyl compounds and their precursors and (3) to identify carbonyl compounds by non-target screening combined with kinetic and mechanistic information.

2. Materials and methods

2.1. Reagents and solutions

A list of chemicals, solutions and water types which were used in this study and information on their purity and suppliers is provided in Tables S1–S4 in Section S1 (supporting information (SI1)).

2.1.1. Lake and wastewater samples

Grab samples were taken from the two lake waters (LW) Lake Greifensee and Lac de Bret, Switzerland and from three secondary treated wastewater effluents (WW) Neugut (Dübendorf), Werdhölzli (Zürich) and Glarnerland (Bilten), Switzerland. The grab samples were used for batch ozonation experiments (Section 2.2). For the wastewater treatment plant (WWTP) Werdhölzli, additionally grab samples were obtained after full-scale ozonation (O_3 plant) and sand filtration (SF plant) for three specific ozone doses (0.27, 0.42, 0.90 mgO_3/mgC). These samples were directly derivatised after filtration (Section 2.3.1). Samples were collected in pre-rinsed plastic (LW Lake Greifensee) or glass bottles, filtered by pre-rinsed 0.45 μm glass fibre filters (Sartorius AG, Goettingen, Germany) and stored at 4 °C until use (between 1–4 days). Water quality parameters of the respective waters are provided in Table S3 (SI1).

2.2. Ozonation of model compounds and different water types

Model compound solutions (phenol, 4-ethylphenol, 4-methoxyphenol, sorbic acid, 3-buten-2-ol and acetylacetone, Section S1, SI1) were ozonated in the presence of DMSO (as $\bullet\text{OH}$ scavenger, 2 mM) with molar ozone:model compound ratios in the range of 0–5 (corresponds to a range of 0–6 mgO_3/mgC , depending on the model compound). Suwannee River Fulvic acid isolate (SRFA), LW and WW samples were ozonated at different specific ozone doses (between 0 and 3 mgO_3/mgC) in laboratory-controlled experiments. Samples either contained phosphate buffer (5 mM) at pH 7.0 ± 0.1 with and without DMSO ($\bullet\text{OH}$ radical scavenger, see below) and were filtered again or were not treated further (Table S4, SI1). Buffer was applied to have comparable ozonation conditions amongst the different water types. Unbuffered samples were necessary to directly compare to full-scale ozonated wastewater with pH changes < 0.3 pH units upon ozonation. Buffered and unbuffered samples were comparable in terms of detected formulas (Section S2, SI1). To determine the effect of $\bullet\text{OH}$ on the formation of carbonyl compounds during ozonation, two sets of samples were ozonated, in presence (0.5 mM) and absence of DMSO (Table S4, SI1). DMSO was chosen as $\bullet\text{OH}$ scavenger because it has much lower yields of formaldehyde (1%) (Yurkova et al., 1999) from the reaction with $\bullet\text{OH}$ compared to *t*-butanol (25–30%) (Acero and von Gunten, 2000; Flyunt et al., 2003; Piechowski et al., 1992).

2.3. Measurement and evaluation of carbonyl compounds

2.3.1. Derivatisation of samples

Model compound solutions were diluted 5–50 times, the other samples (SRFA, LW, WW) from bench-scale ozonation were unchanged. Each sample was spiked with 100 nM benzaldehyde- d_6 (derivatisation efficiency of benzaldehyde 82–98% (Manasfi et al., 2023)) and 32.5 nM tramadol- d_6 before derivatisation as process and instrument controls, respectively. Derivatisation proceeded by the addition of 200 μ M *p*-toluenesulfonylhydrazine (TSH, except model compounds which were derivatised with 50 μ M TSH) and 0.02 M HCl as specified in Manasfi et al. (2023).

2.3.2. LC-ESI-HRMS

Samples were analysed by high-performance liquid chromatography (UltiMate 3000 UHPLC system, Dionex) coupled to a high-resolution hybrid quadrupole-orbitrap mass spectrometer with positive electrospray ionisation (+ESI) (Q Exactive Plus, Thermo Scientific, resolution 140 000 at 200 m/z) as specified in Manasfi et al. (2023) and briefly described in Section S3 (SI1).

2.3.3. Quantification of target carbonyl compounds

The concentrations of acetaldehyde, formaldehyde, 2,3-butanedione and glyoxylic acid in the samples were quantified based on external calibrations of the target carbonyl compounds in ultra-purified water (for details see Manasfi et al., 2023). Samples were blank corrected with ultra-purified water only or containing phosphate buffer (5 mM) with and without DMSO (0.5 mM) measured before and after ozonation (with around 100 μ M O_3) followed by derivatisation. The effect of DMSO on detected areas, limits of quantification (LOQs) and measurement ranges of the four target carbonyl compounds are provided in Fig. S2 and Table S5 (SI1), respectively.

2.3.4. Workflow to obtain molecular formulas from non-targeted carbonyl compounds

A typical workflow is presented in Manasfi et al. (2023). In brief, Compound Discoverer 3.2 (Thermo Scientific, Germany) was used for data analysis by applying the node “Compound Class Scoring” which highlights signature fragments of a derivatised carbonyl compound. Peak areas for pattern analysis and calibration were obtained by Trace Finder 5.1 (Thermo Scientific, Germany). Molecular formulas were obtained by subtracting the molecular composition of TSH from the predicted formula obtained from Compound Discoverer 3.2 using a scripting node (R Studio). An additional scripting node was implemented to obtain the C:O and C:H ratios of each predicted composition. The tentative identification of a carbonyl compound was based on the following three criteria: (i) presence of at least 1 out of 3 signature fragments in the MS^2 spectrum, (ii) 5-fold higher peak area compared to the blank and (iii) 2-fold higher peak area in an ozonated sample compared to a non-ozonated sample. These strict criteria enable to identify carbonyl compounds with high certainty (Manasfi et al., 2023). All peak areas discussed in the text are reported relative to the internal standard benzaldehyde- d_6 -TSH. The analyses outputs from Compound Discoverer and Tracefinder were exported into excel files, where they were further processed in R.

2.3.5. Prioritisation and structural elucidation of carbonyl compounds

Predicted formulas were prioritised based on (1) number of water types with detection of a compound, (2) number of carbons (up to C_5 with a few exceptions) and (3) number of potential structures via Scifinder (Service Chemical Abstracts, 2022). Selected unknown predicted formulas were structurally explained by presuming a carbonyl functional group for each derivatised site and based on the following information: (i) MS^2 fragments and retention time, (ii) formation trend upon ozonation (Section 3.2.1), (iii) influence of ozonation conditions (formation in the presence/absence of $\cdot OH$) (Section 3.2.2), (iv) kinetics and

(v) control experiments with model carbonyl compounds and ozonated DOM model compounds (Section 3.3). Structure assignment is reported by previously defined confidence levels (CL) (Schymanski et al., 2014). Level 3 is the lowest confidence reported in this study because the compound class is clear for all detected molecular formulas based on the observed derivatisation and thus tentative structures could be provided for all detected molecular formulas. 25 commercially available standards were purchased (C_2 - C_5 -carbonyl compounds, Table S1, SI1). A selection of carbonyl compounds was confirmed (CL = 1) by matching the retention time and MS^2 spectrum to these standards (see SI1 for detailed information) which were derivatised and analysed at concentrations between 0.01 – 4 μ M. Additionally, the derivatised standard samples were added to freshly ozonated wastewater to account for matrix effects. No substantial differences in the intensity, ionisability and retention times were observed for different matrices (Manasfi et al., 2023) and in the presence of DMSO (Fig. S2, SI1).

2.3.6. Kinetic information

There are virtually no second-order rate constants for the reactions between ozone and the reported carbonyl compounds. Thus, second-order rate constants for olefins were estimated based on the QSAR presented in Lee and von Gunten, 2012. Often, the second-order rate constant of the corresponding acid was taken, because of the similar Taft constants for CHO vs. CO_2H (2.15 and 2.08), respectively.

2.4. Supporting information (SI)

Three files containing SI were generated: SI1 contains background information to analytical, modelling and mechanistic data. SI2 summarises 36 non-target carbonyl compounds with (a) measurement information and MS^2 spectra, (b) formation trends upon ozonation, (c) comparison of formation for different water types, (d) formation trends from precursors, if applicable, and (e) ion chromatograms or further MS^2 spectra, if applicable. The information is numbered and referenced as SI2.1–36(a-e), respectively. SI3 is an excel file containing all detected non-target carbonyl compounds from the different water types (sheet 1) and precursors (sheet 2), summarising the information discussed below.

3. Results and discussion

3.1. Detection of carbonyl compounds in ozonated lake waters and wastewaters

3.1.1. Overview and characteristics of the formed carbonyl compounds

Three wastewater effluent and two lake water samples were ozonated in bench-scale experiments followed by derivatisation with TSH. In addition, full-scale ozonated WWTP samples were derivatised directly after sampling. Predicted molecular compositions were obtained from these samples as described elsewhere (Manasfi et al., 2023) and briefly outlined in Section 2.3.4. In total, 178 unique molecular formulas for carbonyl compounds were annotated with the number of carbons ranging from C_1 to C_{18} and the highest frequency between C_4 and C_6 (Figs. S5a and c, SI1). On average, 91% of the formulas contain at least two out of three signature fragments from the derivatising agent in the MS^2 spectra (Fig. S5b, SI1). Amongst the carbonyl compounds, 14 formulas (8%) are also detected before ozonation and 57 formulas (32%) contain at least one nitrogen atom (see SI3 for all carbonyl compounds). 116 of the 178 formulas (65%) were detected in > 2 water types (SI3). Further details on compound confirmation, including the elucidation of isomers, is provided in Section 3.4 and SI2.

The predicted formulas containing a carbonyl functional group formed upon ozonation are visualised in a van Krevelen diagram in Fig. 1a which shows the ratios of H:C versus O:C for each predicted formula. Formulas which were detected more frequently are designated with larger circle sizes. The blue lines (square for aromatic compounds) indicate the presence of carbonyl compounds belonging to groups 1 – 5

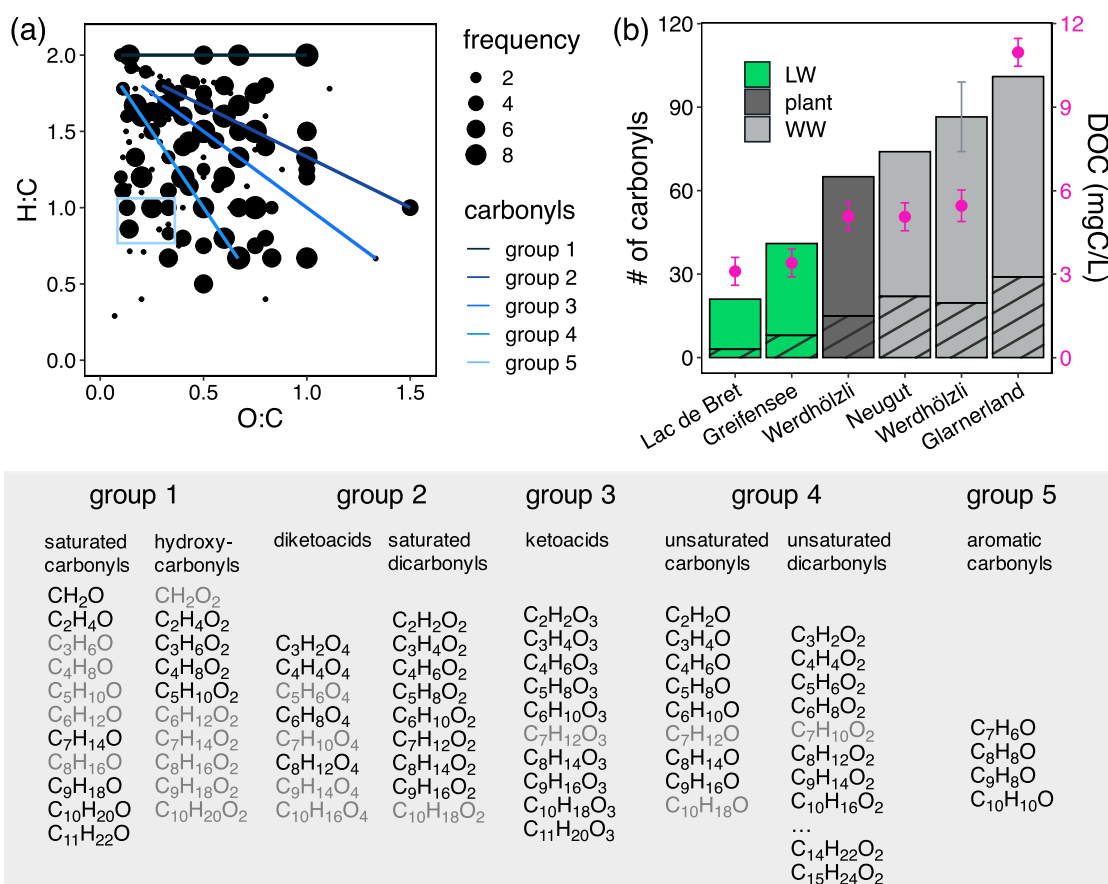


Fig. 1. (a) van Krevelen diagram of carbonyl compounds formed during ozonation of different water types. Frequency indicates the number of water types in which a formula was detected (the maximum of 8 corresponds to water types without DMSO as presented in Table S4, SI1). Groups 1–5 indicate the series of carbonyl compounds as indicated by the molecular formulas (black formulas were detected in this study). (b) Bar chart of number of carbonyl compounds detected in the respective water types by the applied workflow. Striped stacks indicate the number of *N*-containing carbonyl compounds. The corresponding DOC concentrations are shown on the secondary y-axis (pink).

(lower panel Fig. 1). Detection of carbonyl compounds in these 5 groups include saturated, hydroxylated, unsaturated (di)carbonyls, keto- and diketoacids and aromatic carbonyl compounds (indicated by black font). The observation is coherent with previous studies that reported a wide range of carbonyl compounds formed during ozonation (Hammes et al., 2006; Liu et al., 2020; Prasse et al., 2018; Richardson et al., 1999; Świątlik et al., 2004; Wert et al., 2007). Amongst the carbonyl molecular formulas displayed in Fig. 1a, also *N*-containing formulas were detected, which cover the whole H:C to O:C range (Fig. S6b, SI1).

Compared to formulas typically detected before ozonation (region of grey box, Fig. S6a, SI1), Remucal et al., 2020 have shown a shift to highly oxidised formulas upon ozonation of DOM (green box in Fig. S6a, SI1) (i.e. from O:C ratios < 0.5 to the range of 0.5–1.5 with constant H:C ratios). Liu et al., 2020 observed a similar shift for the formed carbonyl compounds but at generally lower O:C ratios from ≥ 0.2 to ≤ 1 (pink box, Fig. S6a, SI1). These differences might be caused by a detection of only derivatised carbonyl compounds compared to all features in Remucal et al., 2020. Formulas at O:C > 1 were only detected in the study of Remucal et al., 2020. Therefore, formulas at O:C > 1 must be other compound classes such as carboxylic acids which are not derivatised and hence not detectable in Liu et al., 2020 and in the present study. Fig. 1a also shows many carbonyl compounds at O:C < 0.5 in line with Liu et al., 2020, but in contrast to past findings also H:C ratios ≤ 1.5 are observed (Fig. S6a, SI1). This observation is reasonable if carbonyl compounds detected in this region are poorly retained in typical sample preparation procedures involving solid phase extraction (Liu et al.,

2020) and poorly ionised during electrospray ionisation (Remucal et al., 2020). Thus, derivatisation of carbonyl compounds without prior sample treatment leads to a detection of additional compounds and complements previous findings.

3.1.2. Comparison of carbonyl compounds formed during ozonation of different water types

Before comparing different water types, the reproducibility of detection of carbonyl compounds in the same water type was evaluated. The reproducibility is illustrated by an ozonated secondary wastewater effluent from Werdhölzli for the same day and for different experimental conditions (Table S4, SI1). This comparison shows an overlap of > 80% of the annotated formulas amongst samples, for formulas with ≤ 6 carbon atoms (Section S2, Fig. S1, SI1). Consequently, differing experimental conditions (i.e. buffered vs. non-buffered or in the presence or absence of an •OH scavenger), do not lead to a significant change in detection of carbonyl compounds. A general comparison reveals that the number of carbonyl-containing formulas detected after ozonation of wastewater is higher than in lake water (Fig. 1b). A major influence for this finding is the higher DOC concentration (secondary y-axis in Fig. 1b) in wastewater, which may lead to higher concentrations and enhance the detectability of carbonyl compounds to a certain extent. A comparison of carbonyl compounds formed in the different water types reveals the formation of an overlapping subset of carbonyl compounds (20%, Tables 1 and 3 and SI3). Tables 1 and 3 present the annotated formulas for a selection of carbonous and nitrogenous carbonyl

Table 1

Selected carbonous carbonyl compounds (complete list in S13) detected in different water types. The formulas are sorted by increasing number of carbons from top to bottom.

Formula of carbonyl compound	(tentative) name	confidence level	formation trend	number of derivatised sites	accurate mass of derivatised form	retention time (min)	Lake Greifensee	Lac de Bret	WWTP Glarnerland	WWTP Neugut	WWTP Werdhölzli	Werdhölzli plant	SRFA	precursors ^a
CH ₂ O	formaldehyde ^b	1	(i)	1	198.0460	13.4	✓	✓	✓	✓	✓	✓	✓	✓
C ₂ H ₂ O ₂	glyoxal ^b	1	(iv)	1	394.0767	16.4	(✓)	(✓)	✓	✓	✓	(✓)	✓	✓
C ₂ H ₂ O ₃	glyoxylic acid ^b	1	(iv)	1	242.0357	13.4	(✓)	(✓)	✓	✓	✓	✓	(✓)	✓
C ₂ H ₄ O	acetaldehyde ^b	1	(ii)	1	212.0617	13.8		✓	✓	(✓)	✓	✓	✓	✓
C ₂ H ₄ O ₂	hydroxyacet-aldehyde	1	(i)	1	228.0569	12.7	✓	(✓)	✓	✓	✓	✓	✓	✓
C ₃ H ₂ O		3	(i)	1	222.0463	12.7			✓	✓	✓	✓	(✓)	✓
C ₃ H ₂ O ₂	3-oxo-2-propenal	2	(iv)	1-2	238.0410	14.8	✓	✓	✓	✓	✓	✓	✓	✓
C ₃ H ₂ O ₃	2-oxo-propanedial	2	(iv)	2	422.0721	16.6	(✓)	(✓)	✓	✓	✓	✓	✓	✓
C ₃ H ₄ O	acrolein	1	(v)	1	224.0622	16.1		(✓)	✓		✓	(✓)		
C ₃ H ₄ O ₂	malondialdehyde	1	(iv)	2	408.0927	14.7			(✓)	(✓)	(✓)	(✓)		
C ₃ H ₄ O ₂	methyl glyoxal	1	(iv)	2	408.0927	16.4			✓	✓	(✓)	(✓)	✓	✓
C ₃ H ₄ O ₃	pyruvic acid ^b	1	(iv)	1	256.0514	13.8	✓	(✓)	✓	✓	✓	✓	✓	✓
C ₃ H ₄ O ₃	hydroxy-oxo-propanal	3	(v)	2	424.0873	16.4	(✓)		✓	✓	(✓)	(✓)	✓	
C ₃ H ₆ O ₂	hydroxyacetone	1	(i)	1	242.0726	12.9	✓	(✓)	✓	✓	✓	✓	✓	✓
C ₄ H ₂ O ₂		3	(iv)	1	250.0416	12.8	✓	(✓)	✓	✓	✓	(✓)		
C ₄ H ₄ O		3	(ii)	1	236.0617	13.6	✓	(✓)	✓	✓	✓	✓		
C ₄ H ₄ O ₂	2-butene-1-4-dial	1	(iv)	1	252.0567	13.3	(✓)	✓	(✓)	(✓)	✓	✓	(✓)	✓
C ₄ H ₄ O ₂	C ₄ -furanone	3	(iv)	1	252.0566	15.7	✓	(✓)	✓	✓	✓	✓	✓	✓
C ₄ H ₄ O ₃		3	(i)	1	268.0513	13.4	✓	✓	✓	✓	✓	✓	✓	
C ₄ H ₄ O ₃	4-oxobut-3-enoic acid	2	(ii)	1	268.0517	14.9	(✓)	(✓)	✓	(✓)	✓	(✓)	(✓)	✓
C ₄ H ₄ O ₃	4-oxo-3-hydroxy-butanal	2	(ii)	1	268.0518	14.2	(✓)	(✓)	✓	✓	✓	✓	✓	✓
C ₄ H ₆ O ₂	2,3-butanedione ^b	1	(iii)	1	254.0722	15.7	✓	✓	✓	✓	✓	✓	✓	✓
C ₄ H ₆ O ₄		3	(i)	1	286.0625	12.7	(✓)	(✓)	✓	✓	✓	✓	✓	✓
C ₄ H ₆ O ₃	3/4-oxobutanoic acid	3	(iv)	1	270.0671	13.7	(✓)	(✓)	✓	✓	✓	✓	✓	✓
C ₅ H ₄ O ₂		3	(iv)	1	264.0568	12.7	(✓)	(✓)	✓	(✓)	✓	✓	(✓)	
C ₅ H ₄ O ₃		3	(iv)	1	280.0514	13.9	✓	(✓)	✓	✓	✓	✓	(✓)	✓
C ₅ H ₄ O ₃		3	(iv)	1	280.0518	16.5	(✓)	(✓)	(✓)	(✓)	✓	✓	✓	
C ₅ H ₄ O ₄		3	(iii)	1	296.0470	12.8	(✓)	(✓)	✓	✓	✓	(✓)	(✓)	
C ₅ H ₆ O		3	(ii)	1	250.0773	14.4	✓	(✓)	✓	✓	✓	✓	(✓)	✓
C ₅ H ₆ O ₂	4-oxo-2-pentenal	2	(ii)	1	266.0725	14.1	✓	✓	✓	✓	✓	✓	(✓)	
C ₅ H ₆ O ₃	4-oxo-2-pentenoic acid	1	(ii)	1	282.0674	14.3	(✓)	(✓)	✓	✓	✓	(✓)	✓	✓
C ₅ H ₆ O ₃	2,3,4-pentanetrione or furancarbox-aldehyde isomer	3	(iv)	2	450.1030	16.5	(✓)		✓	✓	✓	✓	(✓)	
C ₅ H ₈ O	cyclopentanone	3	(i)	1	252.0930	16.9	✓	(✓)	✓	✓	✓	✓	✓	
C ₅ H ₈ O ₂	glutaraldehyde ^b	1	(ii)	1	268.0882	14.5	(✓)	(✓)	✓	✓	✓	✓	(✓)	✓
C ₅ H ₈ O ₂	2,3-pentanedione	1	(i)	1	268.0884	16.7	(✓)	(✓)	(✓)	(✓)	✓	✓	(✓)	✓
C ₅ H ₈ O ₃	3-methyl-2-oxo-butanolic acid	3	(ii)	1	284.0831	13.8	✓	✓	✓	✓	✓	✓	✓	
C ₆ H ₆ O ₃	6-oxo-2,4-hexadienoic acid	3	*	1	294.0674	14.0			✓	(✓)	✓	✓		
C ₆ H ₆ O ₃	hydroxy-muconaldehyde	3	(iii)	1	294.0674	14.7			✓	(✓)	✓	✓	✓	✓

^aprecursors are: phenol, 4-ethylphenol, 4-methoxyphenol, sorbic acid, 3-buten-2-ol, acetylacetone, ^bcompound was detected with the same method and quantified in [Manasfi et al. \(2023\)](#). Tick in brackets: a peak with this mass and retention time was detected but not detected by our workflow (peak area between 1-2 times higher in ozonated sample vs. non-ozonated sample or MS² was too weak or absent). Formation trends: (i) primary with constant maximum, (ii) primary with further abatement, (iii) products with primary trend but less efficient formation, (iv) secondary product with constant maximum and (v) secondary product with further abatement (see [Fig. 2](#) for examples).

compounds, respectively. The selection includes the subset of carbonyl compounds detected in all water types, additionally to those detected in all wastewaters and acrolein and $C_4H_5NO_2$ mentioned in the text (for all carbonyl compounds refer to SI3). Amongst the detected carbonyl compounds, $17 \pm 3\%$ and $30 \pm 1\%$ contain at least one nitrogen atom in lake water and wastewater, respectively (the number of N-containing carbonyl compounds in each water type is shown by the striped stacks in Fig. 1b). Thus, a significantly higher fraction of N-containing carbonyl compounds is detected in wastewater compared to lake water (Fig. S7, SI1), which is rationalised by higher concentrations of nitrogen-containing biomolecules in wastewater compared to lake water. Past studies showed increased detection of CHON formulas in the lipid- and protein-like regions in traditional van Krevelen diagrams in wastewater compared to natural aquatic systems and downstream of an increasingly wastewater impacted river (Berg et al., 2019; Maizel and Remucal, 2017).

The strict criteria (factor 5 higher than blank, factor 2 higher than non-ozonated sample and presence of signature fragments in the MS² spectra) enabled the detection of a carbonyl compound with high certainty, but some detected carbonyl compounds with lower certainty were rejected (such compounds have a bracket around the tick in Tables 1 and 3).

3.2. Formation of carbonyl compounds upon ozonation

Monitoring the formation trends of carbonyl compounds as a function of the specific ozone doses in the different water types in presence or absence of DMSO adds kinetic and mechanistic information to each molecular formula.

3.2.1. Formation trends and kinetic information

In the following section, typical trends upon ozonation are discussed for target and non-target carbonyl compounds (Fig. 2a and c). The target carbonyl compounds were selected based on a companion study, where they are detected and quantified in the range of the applied ozone doses in different water types and discussed in more detail (Manasfi et al., 2023). Here, four of these target carbonyl compounds serve as examples for different trends, which are discussed exemplarily to assess the observed trends for non-target carbonyl compounds. An overview of trends for selected molecular formulas is provided in Tables 1, 3 and for all molecular formulas in SI3. Fig. 2a and c show these selected target and non-target carbonyl compounds with five commonly observed formation trends for a bench-scale ozonated secondary effluent from WWTP Werdhölzli. If detected, the compounds usually show similar formation trends in all water types including full-scale ozonated secondary wastewater effluent (Figs. S8–S11, SI1).

- (i) *Primary products with constant maximum.* Please note, “primary” in this context is used for products with an immediately increasing trend as a function of the ozone doses, but not necessarily to describe a primary product from a precursor in the water matrix. Formaldehyde (CH_2O , pink diamonds, Fig. 2a) is relatively efficiently formed from multiple precursors. Precursors responsible for formaldehyde formation are never fully depleted and therefore, a steady-state concentration of formaldehyde is reached resulting from its continuous slow formation from precursors and slow abatement by ozone and $\bullet OH$ ($k_{O_3} = 0.1 M^{-1}s^{-1}$ (Hoigné and Bader, 1983), $k_{OH} \sim 10^9 M^{-1}s^{-1}$ (Buxton et al., 1988)) at higher specific ozone doses.
- (ii) *Primary products with further abatement.* Acetaldehyde formation is shown as a function of increasing specific ozone doses in Fig. 2a (C_2H_4O , black circles). The concentration reaches a maximum at typically applied specific ozone doses for enhanced wastewater treatment ($0.5 - 1 mgO_3/mgC$ (Bourgin et al., 2018)), and decreases for higher ozone doses. Its reactivity with ozone ($k_{O_3} = 1.5 M^{-1}s^{-1}$ (Hoigné and Bader, 1983) and $\bullet OH$ ($k_{OH} = 2.4 \cdot 10^9$

$M^{-1}s^{-1}$ (Schuchmann and von Sonntag, 1988)) is low, but an $\bullet OH$ -induced abatement seems still plausible, when the precursors are fully abated at higher specific ozone doses $> 1 mgO_3/mgC$.

- (iii) *Products with direct but less efficient formation.* 2,3-butanedione ($C_5H_6O_2$, green squares, Fig. 2a) is an example of a product that is continuously formed from precursors in DOM that react with intermediate second-order rate constants with ozone ($k_{O_3} \sim 10^3 M^{-1}s^{-1}$ (Lim et al., 2022; von Sonntag and von Gunten, 2012)). Thus, the concentrations of such precursors are only partially depleted, and therefore, the compound continuously increases for all applied specific ozone doses.
- (iv) *Secondary products with constant maximum.* A lag-phase in the increase as a function of the specific ozone dose is typical for secondary ozonation products. Glyoxylic acid ($C_2H_2O_3$, blue triangles, Fig. 2a) shows this trend which is expected based on its known formation pathway as end product from multiple ozone attacks on phenolic precursors (Ramseier and von Gunten, 2009). For non-target carbonyl compounds, similar trends were observed (Fig. 2c). Additionally, another trend was commonly observed, which was not clearly seen for target carbonyl compounds (Manasfi et al., 2023).
- (v) *Secondary products with further abatement.* The additional formation trend includes secondary products which show the same lag-phase as trend (iv) but followed by further abatement. An example for this trend is $C_4H_7NO_3$ (grey reverse triangles in Fig. 2c, SI2.35 and Table 3).

For non-target carbonyl compounds, formation trends can provide information about their precursors and their reactivities with O_3 and/or $\bullet OH$. Examples are discussed in Sections 3.2.2, 3.3 and 3.4.

Besides the formation trend of a carbonyl compound upon ozonation, in some cases also the stability of a derivatised compound influences its observed fate, which can be used as additional information about the compound. The measurements of glyoxylic acid in Fig. S10 (SI1) are quite scattered (measurement sequences: e.g. sample 1 at all ozone doses followed by sample 2 at all ozone doses, decrease in peak areas over the measurement time). A previous study reported that derivatised ketoacids are unstable (Siegel et al., 2014). Consequently, scattered trends may imply the presence of an acid moiety (i.e. Figs. SI2.S23b or SI2.14b) and if aimed to quantify, such compounds should be assessed for their stabilities as evaluated and discussed in Manasfi et al. (2023).

3.2.2. Role of water type and mechanistic aspects

In general, the concentrations of the formed carbonyl compounds are lower in lake water compared to wastewater, which is reflected in Fig. 2b for a specific ozone dose of $1 mgO_3/mgC$ (similar trends at $0.5 mgO_3/mgC$, data not shown). Concentrations as a function of ozone doses for a selection of target carbonyl compounds in different water types are discussed in detail elsewhere (Manasfi et al., 2023). To obtain a better understanding of the formation of unknown carbonyl compounds in different water types, ozone dosage experiments were performed with SRFA and different water types in the presence of DMSO, a $\bullet OH$ scavenger. Additionally, the concentrations or areas were DOC concentration-normalised to reduce possible trends caused by this difference.

From the carbonyl compounds evaluated in this study, three classes can be distinguished (Fig. 3a). The carbonyl compound yields in presence compared to absence of an $\bullet OH$ scavenger are (1) higher (Fig. 3a, green), (2) lower (Fig. 3a, blue) or (3) similar (Fig. 3a, pink) when DOC concentration-normalised areas are used.

(1) Higher areas in the presence of a scavenger are expected from precursors with high ozone reactivity and ensuing carbonyl compound formation (Fig. 3a, green or Fig. SI2.8c). The carbonyl compounds are lower in the absence of a scavenger because their precursors could also react to other products in the presence of $\bullet OH$. (2) If lower areas are

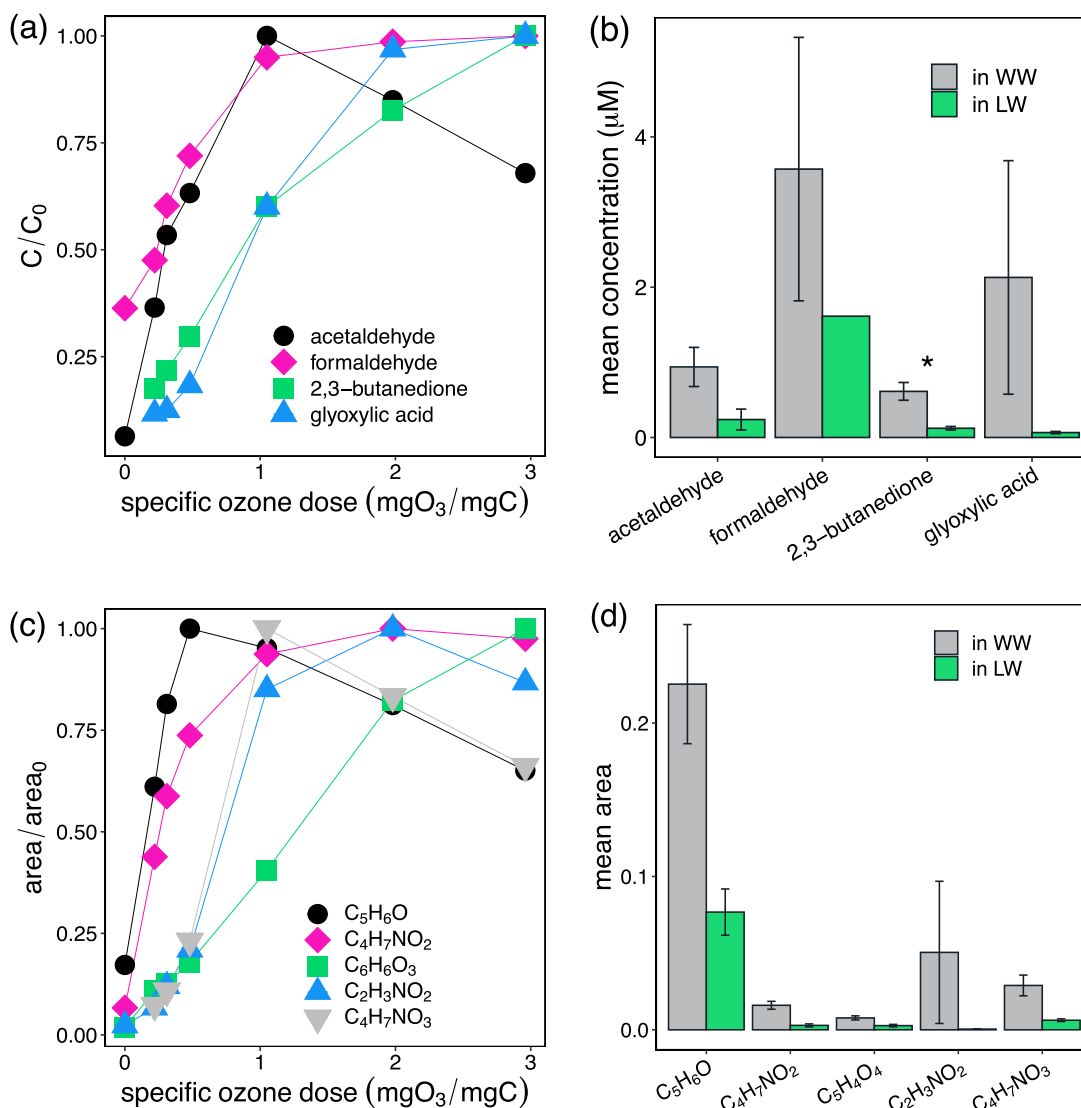


Fig. 2. (a, c) Evolution of the relative concentrations or relative peak areas of four target carbonyl compounds or non-target carbonyl compounds, respectively, as a function of the specific ozone doses for Werdhölzli wastewater ($n = 6$). (b, d) Mean concentrations or mean peak areas of the same target carbonyl compounds or non-target carbonyl compounds, respectively, for a specific ozone dose of 1 mgO_3/mgC for wastewater (Neugut, Werdhölzli and Glarnerland, grey) and lake water (Lac de Bret and Lake Greifensee, green), respectively (*concentrations of 2,3-butanedione were multiplied by 5 for better readability). Error bars in a and c were omitted for better readability but replicates are shown for the individual target compounds in Figs. S8–S11, SI1. Error bars in b and d represent the standard deviations for the different water types.

observed in presence of a scavenger (Fig. 3a, blue or Figs. SI2.4,6,12,14–16c), two possible explanations exist: (i) The carbonyl compound shows a relatively high k_{O_3} and because the presence of an $\bullet\text{OH}$ scavenger leads to a higher lifetime of ozone, an enhanced abatement of the carbonyl compound is expected. (ii) The carbonyl is formed by independent reactions with different precursors directly with ozone and/or $\bullet\text{OH}$. Therefore, the DOC concentration-normalised area is higher in the absence of a scavenger when the pool of precursors is larger due to independent contributions from both O_3 and $\bullet\text{OH}$. This case is expected if the formed carbonyl compound has a low k_{O_3} . (3) Similar areas in the presence and absence of a scavenger can be obtained by a complex balance of formation and abatement of carbonyl compounds by O_3 and $\bullet\text{OH}$ (Fig. 3a, pink or Figs. SI2.7,21,33–35c).

A comparison of the selected four target carbonyl compounds reveals that formaldehyde and glyoxylic acid are formed at much higher concentrations in presence of DMSO. In contrast, acetaldehyde and 2,3-

butanedione yield higher concentrations in absence of DMSO (Figs. S8b–S11b, SI1). Formaldehyde and glyoxylic acid are main byproducts from direct ozonation reactions (von Sonntag and von Gunten, 2012), while the observations suggest that reactions with both O_3 and $\bullet\text{OH}$ may have led to the formation of acetaldehyde and 2,3-butanedione.

For some carbonyl compounds detected in a lake water, the DOC concentration-normalised peak areas for a specific ozone dose of 1 mgO_3/mgC are similar to wastewater, indicating that such carbonyl compounds are formed independently of the water type ($\text{C}_4\text{H}_4\text{O}_3$ in Fig. 3b or e.g. Figs. SI2.14–15c). However, most of the carbonyl compounds show a substantially higher DOC concentration-normalised peak area in wastewater, indicating an origin from precursors which are more abundant in wastewater ($\text{C}_3\text{H}_5\text{NO}_2$ in Fig. 3b and e.g. Figs. SI2.2,11,33–34c). Similar trends are generally observed for non-target carbonyl compounds and thus this information will be used in Section 3.4 for the discussion of identified non-target carbonyl compounds.

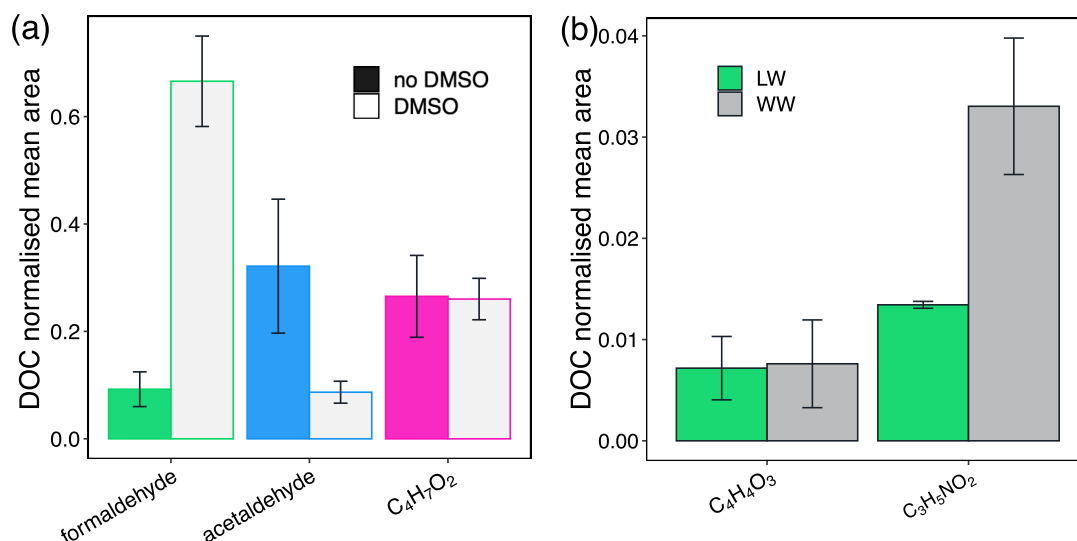


Fig. 3. (a) DOC concentration-normalised areas of carbonyl compounds in the presence (full, coloured bars) and absence (light grey bars) of an $\cdot OH$ scavenger (DMSO, 0.5 mM) in laboratory ozonated wastewater (Werdhölzli). Three cases; green = higher, blue = lower, pink = equal in the presence of a scavenger. Mean areas of $C_4H_7O_2$ were multiplied by 100 for better readability. (b) DOC concentration-normalised mean areas of two carbonyl compounds ($C_4H_4O_3$; extent of formation independent of water type, $C_3H_5NO_2$; extent of formation higher in wastewater) in ozonated lake water and wastewater. Conditions: specific ozone dose: 1 ± 0.1 mgO_3/mgC (as specified in Table S4, SI1).

3.2.3. Fate of carbonyl compounds during biological post-treatment

The formation trends discussed above indicate a general increase of carbonyl compounds upon ozonation. Typically, ozonation is followed by a biological post-treatment (Gulde et al., 2021; Hammes et al., 2006; Krasner, 2009; Philip Singer, 1994; van der Kooij et al., 1989; Völker et al., 2019). Full-scale ozonation at the WWTP Werdhölzli is followed by biological sand filtration, and the fate of the non-target carbonyl compounds was evaluated after this process. It was demonstrated, that the mean areas of non-target carbonyl compounds are abated during biological sand filtration almost to the levels prior to ozonation (Manasfi et al., 2023). Herein, the fate is shown for a selection of carbonyl compounds (Fig. S18, SI1, Section 3.4).

3.3. Link between detected carbonyl compounds and potential precursors

Previous studies showed, that electron-rich moieties in the water matrix and especially phenols belong to the main ozone consumers and DBP precursors during ozonation (Houska et al., 2021; Önnby et al., 2018; von Gunten, 2018; Walpen et al., 2020). To link typical precursors to the formation of carbonyl compounds, six model precursors; phenol, 4-ethylphenol, 4-methoxyphenol, sorbic acid, 3-buten-2-ol and acetylacetone and further Suwannee River Fulvic acid (SRFA) were ozonated.

Table 2 provides an overview of carbonyl compounds formed upon ozonation from the selected model precursors (all formed carbonyl compounds are provided in SI3). The size of the circles provides an estimate for the extent to which a carbonyl compound is formed from a precursor based on its measured peak area. The formation trends of these carbonyl compounds are provided in Figs. S12–S14, SI1 and in SI2 (if related to non-target carbonyl compounds).

Information obtained in experiments with defined precursors can be used to assess the potential origin of carbonyl compounds. As shown in Table 2 carbonyl compounds with the formula CH_2O (formaldehyde), $C_2H_2O_3$ (glyoxylic acid) and $C_3H_4O_2$ (methyl glyoxal) are formed from all precursors and thus represent unspecific compounds which are also typical end members of ozonation. The formation trends of these compounds upon ozonation of real water samples reflect this observation (Table 1, Fig. 2). Interestingly, many common carbonyl compounds are formed from the three phenolic compounds and sorbic acid (first 10 entries in Table 2). The formation trend is often similar for the three phenols but different for

sorbic acid (Fig. S12, SI1). The formulas C_3H_2O and C_5H_6O contain isomers which formed upon ozonation with mainly phenolic precursors. Their formation seems quite wastewater specific (Figs. SI2.2c and SI2.21c) and independent of the presence of an $\cdot OH$ scavenger.

C_2H_4O (acetaldehyde), is only formed from 4-ethylphenol and 3-buten-2-ol with two distinct formation trends (Fig. S12, SI1). For 3-buten-2-ol ozonation, acetaldehyde is very efficiently formed, which is rationalised by a direct ozonation reaction of a resonance form of 3-buten-2-ol. For 4-ethylphenol ozonation, acetaldehyde is formed less efficiently, suggesting multiple reaction steps leading to its formation.

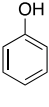
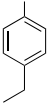
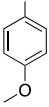
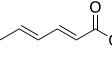


Some isomers of $C_4H_4O_2$ are formed from phenolic but also olefinic precursors, with different formation trends (Fig. S13, SI1). This might be useful to distinguish potential formulas for the different isomers. $C_4H_6O_2$ (2,3-butanedione) is only formed from the olefinic precursors 3-buten-2-ol and acetylacetone. Further oxidation products related to precursors are discussed in Section 3.4 to support structural elucidation of non-target carbonyl compounds.

Role of electron donating groups. Results from precursor ozonation confirm, that many carbonyl compounds are formed from phenol- and β -diketone-type structures (Aeschbacher et al., 2012; Chon et al., 2015; Houska et al., 2021; Önnby et al., 2018; Rougé et al., 2020a,b; Walpen et al., 2020). Oxidation leads to an abatement of the electron donating capacity (EDC), which seems to be a promising link between precursors and DBPs (Aeschbacher et al., 2012; Chon et al., 2015; Houska et al., 2021; Önnby et al., 2018; Rougé et al., 2020a, b; Walpen et al., 2020). Fig. S4 (SI1) shows a linear correlation between the logarithmic area of carbonyl compounds and the measured residual EDC of wastewater samples. The latter highlights, that the EDC is a potential surrogate parameter for the formation of carbonyl compounds upon ozonation.

Nitrogenous carbonyl compounds. No *N*-containing precursors were ozonated and thus only a comparison between the selected water types and ozonated SRFA was possible. Around 15% of *N*-containing carbonyl compounds were detected upon ozonation of SRFA and most of them were also detected in lake water and wastewaters (SI3). This fraction of nitrogenous carbonyl compounds is similar to lake waters (17%) but significantly lower than for wastewaters (30%), in line with the low nitrogen content of SRFA (IHSS, 2020). Consequently, a significant fraction of the nitrogenous formulas detected in the wastewaters might be wastewater specific.

Table 2

Selected carbonyl compounds formed upon ozonation (in the presence of DMSO) of the six model precursors phenol, 4-ethylphenol, 4-methoxyphenol, sorbic acid, 3-buten-2-ol and acetylacetone with indication of the formula and retention time (min). Circle sizes represent the relative peak area intensity of the formed carbonyl compound from a precursor (note that peak area and concentration might not be proportional): ● 1–10, ● 10–100, ● 100–1000, the extent of formation is reported relative to the smallest area of a respective carbonyl compound from an ozonated model compound. Colour of circles (black, blue and green) indicate differences in formation trends for the same carbonyl compound. The respective formation trends for the carbonyl compounds belonging to the molecular formulas are shown in Figs. S12–14, SI1.

formula of carbonyl compound	(tentative) name	retention time (min)						
CH ₂ O	formaldehyde	13.4	●	●	●	●	●	●
C ₂ H ₂ O ₂	glyoxal	16.4	●	●	●	●	●	●
C ₂ H ₂ O ₃	glyoxylic acid	13.4	●	●	●	●	●	●
C ₂ H ₄ O	acetaldehyde	13.8	●	●	●	●	●	●
C ₂ H ₄ O ₂	hydroxyacetaldehyde	12.7	●	●	●	●	●	●
C ₃ H ₂ O		12.7	●	●	●	●	●	●
C ₃ H ₂ O ₂	3-oxo-2-propenal	14.8	●	●	●	●	●	●
C ₃ H ₂ O ₃	2-oxo-propanedial	16.4	●	●	●	●	●	●
C ₃ H ₄ O ₂	methyl glyoxal	16.4	●	●	●	●	●	●
C ₃ H ₄ O ₃	pyruvic acid	13.8	●	●	●	●	●	●
C ₃ H ₄ O ₃		14.8	●	●	●	●	●	●
C ₃ H ₆ O ₂	lactaldehyde	13.6	●	●	●	●	●	●
C ₃ H ₆ O ₂	hydroxy-acetone	12.9	●	●	●	●	●	●
C ₄ H ₄ O ₂	2-butene-1,4-dial	13.3	●	●	●	●	●	●
C ₄ H ₄ O ₂	C ₄ -furanone	15.7	●	●	●	●	●	●
C ₄ H ₄ O ₃	4-oxo-3-hydroxy-butanal	14.2	●	●	●	●	●	●
C ₄ H ₄ O ₃	4-oxobut-3-enoic acid	14.9	●	●	●	●	●	●
C ₄ H ₆ O ₂	2,3-butanedione	15.7	●	●	●	●	●	●
C ₄ H ₆ O ₃	3/4-oxobutanoic acid	13.7	●	●	●	●	●	●
C ₄ H ₆ O ₄		12.7	●	●	●	●	●	●
C ₅ H ₄ O ₃		13.9	●	●	●	●	●	●
C ₅ H ₆ O		14.4	●	●	●	●	●	●
C ₅ H ₈ O ₂	glutaraldehyde	14.5	●	●	●	●	●	●
C ₅ H ₈ O ₃		14.0	●	●	●	●	●	●
C ₆ H ₆ O ₃	6-oxo-2,4-hexadienoic acid	15.6	●	●	●	●	●	●
C ₆ H ₆ O ₃	hydroxy-muconaldehyde	14.7	●	●	●	●	●	●

3.4. Identification of selected carbonyl compounds

In this study, some unknown carbonyl compounds formed during ozonation were identified. Overall, 12 compounds were present, which are amongst target compounds in a companion study (8 > LOQ, 4 < LOQ, Manasfi et al., 2023), 8 compounds were confirmed with CL = 1, and 7 compounds with CL = 2 according to the classification by Schymanski et al., 2014. Moreover, the structure for 6 compounds, which are

classified as CL = 3, can be limited to 2 isomers (SI2). Generally, derivatisation by TSH did not lead to compound-specific fragments apart from TSH signature fragments, indicating the presence of a carbonyl functional group (Manasfi et al., 2023). Nevertheless, distinctive MS² patterns can be obtained for different carbonyl compounds. The proposed carbonyl compounds were further supported by information on the formation trends during ozonation (Section 3.2.1), influence of ozonation conditions (Section 3.2.2) and available kinetic information (summarised

for each discussed carbonyl compound in SI2.1–36). The proposed structures for carbonous and nitrogenous carbonyl compounds are described in Sections 3.4.1 and 3.4.2, respectively.

3.4.1. Carbonous carbonyl compounds

C₂H₄O₂ (SI2.1). Hydroxyacetaldehyde (CL = 1) is the smallest hydroxylated aldehyde, which was detected in the selected water types (SI2.1). Hydroxyacetaldehyde is efficiently formed upon ozonation and levels off at specific ozone doses > 1.5 mgO₃/mgC. Hydroxyacetaldehyde was also formed during ozonation of phenols and sorbic acid (Table 2 and Fig. SI2.1d). A comparison of the different water types at 1 mgO₃/mgC shows higher concentrations in wastewater and a more efficient formation in the presence of •OH (even for DOC concentration-normalised areas) (Fig. SI2.1c). Its formation during ozonation has been previously reported (Le Lacheur and Glaze, 1996; Liu et al., 2015). A possible pathway was shown with serine, where ozonation (in the presence of a •OH scavenger) and decarboxylation at the amino-carbon leads to hydroxyacetaldehyde. Hence, the higher DOC concentration-normalised areas in wastewaters could be explained by the higher concentrations of amino acids in these waters (< 1 µg/L in surface water compared to ≤ 40 µg/L in wastewater (Dotson and Westerhoff, 2009; Yang et al., 2020)). A higher formation in the absence of a •OH scavenger (DMSO) indicates an important contribution of •OH reactions, which would point to other precursors than serine.

C₃H₂O₃ (SI2.5). For C₃H₂O₃, 2-oxo-propanedial (CL = 2, no standard available) is the only probable structure with two derivatisable sites. This compound is very efficiently formed upon ozonation, especially in plant-ozonated wastewater effluents (Fig. SI2.5b). This compound is also formed from phenols (secondary formation trend) and sorbic acid (primary formation trend) (Table 2 and Fig. SI2.5d).

C₃H₄O (SI2.6). Acrolein (CL = 1) is efficiently formed in wastewater in absence of DMSO (Fig. SI2.6b, c) with decreasing peak areas for higher specific ozone doses (> 1 mgO₃/mgC). Acrolein is not formed from any of the precursors or from SRFA. The significantly more efficient formation of acrolein in absence of DMSO indicates, that acrolein is mainly formed by reactions of precursors with •OH (Fig. SI2.6c and Section 3.2.2). Acrolein itself has an estimated second-order rate constant for the reaction with ozone of about 10³–10⁴ M⁻¹s⁻¹ (Lee and von Gunten, 2012) and a high reactivity with •OH (*k*_{OH} = 7.0·10⁹ M⁻¹s⁻¹ (Buxton et al., 1988)). The area also decreases in the presence of DMSO (data not shown), which is a consequence of the direct reaction with ozone at higher specific ozone doses (>1 mgO₃/mgC). Acrolein concentrations of up to 5 µg/L were reported in ozonated wastewater effluent (1 mgO₃/mgC) previously (Marron et al., 2020). In the current study, concentrations of about 30 µg/L (0.6 µM at 1 mgO₃/mgC) were estimated based on the three concentrations used for identification of the compound (three-point calibration). Acrolein is an electrophilic compound, which can react with nucleophiles in proteins (Lopachin and Gavin, 2014; Prasse, 2021). The presence of acrolein in ozonated wastewaters is problematic as it is significantly more toxic than its saturated analogue propionaldehyde (Skog, 1950). This highlights the importance of biological post-treatment, which mostly reduces the toxicity of ozonated wastewater (Escher et al., 2009; Prasse et al., 2015). Qualitative data from the present study indicate a removal of acrolein during sand filtration (Fig. S18, SI1).

C₃H₄O₂ (SI2.7–8). This molecular formula was detected with two derivatisable sites, at retention times 14.7 and 16.4 min, respectively, with distinct MS² spectra. They could be confirmed with standards with CL = 1 as malondialdehyde and methyl glyoxal.

Malondialdehyde has not been reported during ozonation previously. This compound is mostly formed in wastewater and not significantly in lake and SRFA-containing water and not detected at all from precursor ozonation. Malondialdehyde is a naturally occurring product of lipid peroxidation (Thiele et al., 1997). As ozonation can also induce lipid peroxidation or damage cell walls (Hammes et al., 2007) malondialdehyde might be released and therefore represent an indirect

ozonation product (secondary formation trend Fig. SI2.7b). Malondialdehyde shows a high reactivity towards DNA and forms mutagenic and carcinogenic DNA adducts (Marnett, 1999) and is thus undesired in treated waters. Qualitative observations during biological sand filtration after full-scale ozonation of secondary wastewater effluent indicate that malondialdehyde is mostly removed (Fig. S18, SI1).

Methyl glyoxal has been reported as ozonation byproduct previously (Hammes et al., 2006; Richardson et al., 1999; Świetlik et al., 2004; von Sonntag and von Gunten, 2012; Wert et al., 2007). Methyl glyoxal is formed from many precursors (Table 2 and Fig. SI2.8d) and very prominently from SRFA in the presence of DMSO (Fig. SI2.8c). Qualitative observations during biological sand filtration after full-scale ozonation indicate, that methyl glyoxal is degraded to similar concentrations as prior to ozonation (Fig. S18, SI1).

C₃H₆O₂ (SI2.9). Hydroxyacetone (CL = 1) was detected in all ozonated water types, and analogous to hydroxyacetaldehyde, both •OH and direct ozone pathways lead to its formation (Fig. SI2.9c and Section 3.2.2). It was also detected in small concentrations during acetylacetone ozonation (Table 2 and Fig. SI2.9d). It is a primary-type product, with a slight decrease of the peak for high ozone doses. The reactivity of hydroxyacetone with ozone is quite low (*k*_{O₃} ~ 1 M⁻¹s⁻¹, Lee and von Gunten, 2012) and thus it may further react with •OH, which could explain its slightly decreasing trend for higher specific ozone doses. The molecular formula of hydroxyacetone has also been detected in US drinking waters, but no identification was possible (Khan and Weinberg, 2007). In contrast to the different ozonated water types, an isomer was detected by ozonation of the model precursors acetylacetone, sorbic acid and 3-buten-2-ol (Table 2 and Fig. SI2.9d). Based on the Criegee mechanism of 3-buten-2-ol, the isomer lactaldehyde is formed, which was confirmed by a standard with CL=1 (MS² spectrum, Fig. SI2.9e).

C₄H₄O₂ (SI2.12–13). Several isomers of this molecular formula were detected. Evaluation of their MS² spectra and trends after ozonation indicated two main single derivatised carbonyl compounds at retention times of 13.3 (discussed here, SI2.12) and 15.7 min in all water types (SI2.13). Ozonation of phenol yielded a very similar chromatogram (Fig. SI2.12e) and MS² spectrum as the ozonated wastewater samples (Fig. SI2.12a). Previously, an α,β-unsaturated carbonyl compound with this molecular formula has been detected from phenol oxidation by chlorine or •OH (Prasse et al., 2018, 2020) and during the ozonation of furan (Zoumpoulis et al., 2021). Synthesised 2-buten-1,4-dial (Table S1, SI1) also showed an overlap with the chromatogram and MS² spectrum (Figs. SI2.12a and e, CL = 1), with the presence of three peaks with two distinct MS² spectra. The wastewater samples mainly show the presence of the smallest peak at 13.3 min (Fig. SI2.12e). Further investigations with NMR revealed the presence of a hydrated form of the compound (C₄H₄O₂ + H₂O), which exhibits a neutral H₂O loss in the MS (Fig. SI2.12e, green chromatogram). Thus, the observation of three peaks can be explained by the presence of *cis* and *trans* 2-buten-1,4-dial and a hydrated isomer. Many other dicarbonyls may have similar features. To further elucidate this formula, the different peaks were compared based on their evolution trends during ozonation. Fig. SI2.12f shows that all involved species follow the same formation trend during ozonation of phenol in the presence of DMSO. In wastewater samples (Fig. SI2.12g), the hydrated isomer (main peak detected in wastewater, pink and blue circles) has a distinct trend (remains stable) from the other isomers (decrease at higher ozone doses, grey and green circles) which is rationalised by a different reactivity of the two isomers. Finally, the secondary formation trend during ozonation indicates a quite stable compound (isomer at 13.3 min), which is not further degraded in the range of the applied specific ozone doses (Figs. SI2.12b and g). In addition, the higher areas in samples without •OH scavenging indicate its formation from both ozone and •OH reactions (Fig. SI2.12c). Its potential formation during ozone reactions of phenols is discussed below in the next section.

C₄H₄O₃ (SI2.14–17). Four main isomers with this molecular formula were detected. Fig. 4 shows the formation of one C₄H₄O₃ isomer (blue triangles), presumably 4-oxobut-2-enoic acid (CL = 2, SI2.17) as a

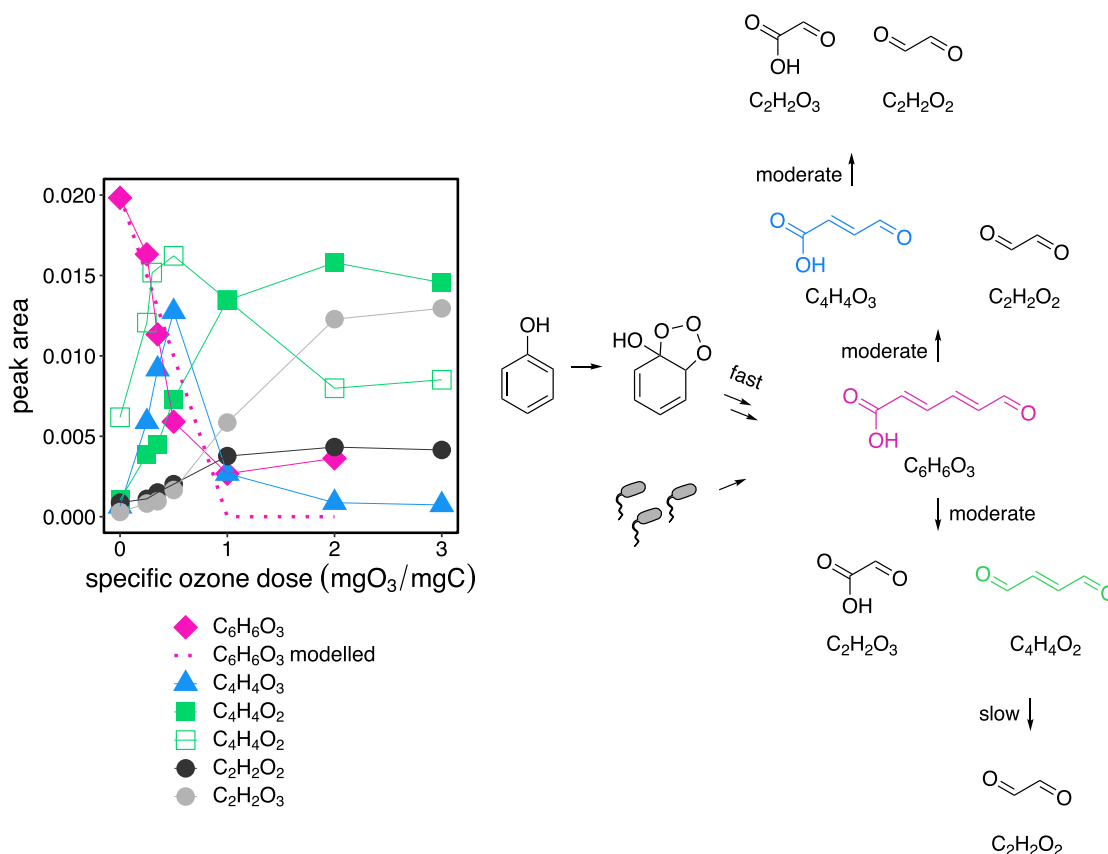


Fig. 4. Example scheme of ozonation of phenolic moieties with the formation of major carbonyl compounds in secondary treated wastewater effluent (Werdhölzli, in the absence of a $\cdot\text{OH}$ scavenger). Left panel: Internal standard corrected peak areas of C₆H₆O₃ (pink diamonds), 4-oxobut-2-enoic acid (C₄H₄O₃, blue triangles), 2-butene-1,4-dial (C₄H₄O₂: filled and empty green squares for two isomers, respectively (peak areas divided by factor of 10 to fit the scale)), glyoxylic acid (C₂H₂O₃: grey circles (peak areas divided by factor of 2 to fit the scale)) and glyoxal (C₂H₂O₂: black squares) upon ozonation of Werdhölzli wastewater (non-treated samples, $n = 6$) as a function of the specific ozone doses (0–3 mgO₃/mgC, error bars were omitted for better readability). C₆H₆O₃ was modelled (dotted pink line) for $c_{\text{C}_6\text{H}_6\text{O}_6} = c_{\text{O}_3} + 10 \cdot c_{\text{O}_3}$ (Section S8, SI1). Right panel: Potential reaction mechanism of phenol transformation upon ozonation, C₆H₆O₃ highlighted in pink, C₄H₄O₃ highlighted in blue and C₄H₄O₂ highlighted in green.

function of the specific ozone doses. After a maximum at a specific ozone dose of 0.5 mgO₃/mgC the compound is significantly abated, indicating a further reactivity with ozone ($k_{\text{O}_3} \sim 10^3 \text{ M}^{-1}\text{s}^{-1}$, similar to fumaric acid (Lee and von Gunten, 2012)). The trend does not significantly differ for the different water samples, but its formation is more pronounced in absence of a $\cdot\text{OH}$ scavenger (Fig. SI2.17c). This carbonyl compound and those discussed in Fig. 4 are all formed during phenol ozonation (Table 2 and Fig. S15, SI1). Furthermore, the MS² spectra recorded in ozonated water samples and phenol solutions overlapped (Fig. SI2.17a). As shown in the right panel of Fig. 4, the formation of C₄H₄O₃ likely results from a muconic acid-type structure, where three isomers (SI2.28–30) were detected in wastewater (here shown: C₆H₆O₃, 6-oxo-2,4-hexadienoic acid (CL = 3), SI2.28) and is a likely product from phenol ozonation (Table 2, Figs. S15 and S16, SI1) (Mvula and von Sonntag, 2003; Ramseier and von Gunten, 2009; Tentscher et al., 2018) or microbial degradation processes of aromatic compounds in secondary treated wastewater effluents (Boyd and Bugg, 2006; Fuchs et al., 2011; Suenaga et al., 2014). As a consequence, this isomer of C₆H₆O₃ was already present in the biologically treated wastewater effluent. The derivatised formula of the proposed structure (C₆H₆O₃, CL = 3, pink diamonds in Fig. 4a and in the right panel) showed a clear decrease during ozonation. This trend is based on a relatively high reactivity with ozone ($k_{\text{O}_3} \sim 10^4 \text{ M}^{-1}\text{s}^{-1}$ similar to muconic acid, Leitzke and von Sonntag, 2009). The dotted pink line represents the modelled decrease of the total C₆H₆O₃ concentration present before ozonation (90%) and formed during ozonation with ensuing oxidation (10%) (von Sonntag and von Gunten,

2012). The decrease coincides with the formation of 4-oxobut-2-enoic acid (C₄H₄O₃, blue triangles) and 2-butene-1,4-dial (C₄H₄O₂, discussed above, filled and empty green squares in Fig. 4).

An ozone attack on muconic acid leads to the formation of 4-oxobut-2-enoic acid (Leitzke and von Sonntag, 2009). A decrease in the peak area for specific ozone doses > 0.5 mgO₃/mgC supports this finding as the deactivated double bond reacts further to glyoxal (C₂H₂O₂, black circles in Fig. 4) and glyoxylic acid (C₂H₂O₃, grey circles in Fig. 4). Such unsaturated ketoacids are likely common transient oxidation byproducts which, due to their moderate ozone-reactivity, may occur at sufficiently high concentrations (in the range of specific ozone doses of 0.5–1 mgO₃/mgC) to yield chlorinated DBPs after post-chlorination (Marron et al., 2020; Marron et al., 2021). Halogenated carbonyl disinfection byproducts from 4-oxobutenoic acid analogues have been reported previously (Liu et al., 2020). The C₄H₄O₂ isomers (filled and empty green squares in Fig. 4) remain quite stable, which may be due to low reactivity or a steady state between formation and abatement, where they react further to glyoxal (C₂H₂O₂, black circles in Fig. 4). As described in SI2.12, C₄H₄O₂ is 2-butene-1,4-dial (CL=1). The presence of such a compound is problematic, because as a type-2-alkene it shows significant toxicity (Lopachin and Gavin, 2014; Prasse, 2021; Prasse et al., 2018). However, qualitative data indicate a removal of 2-butene-1,4-dial during biological sand filtration after ozonation (Fig. S18, SI1).

The exemplary reaction sequence presented in Fig. 4 is based on the assumption of C₆H₆O₃ formation from the phenolic ozonide at positions C₁–C₂. Similar products are potentially formed from other ozonides

(Section S8, SI1) and from muconic acid ($C_6H_6O_4$, not detected in this study), a typical product (6% yield) from phenol ozonation (Ramseier and von Gunten, 2009). More targeted research is needed to corroborate the proposed reaction mechanism, especially including the role of $\bullet OH$.

$C_4H_6O_3$ (SI2.18). This molecular formula has been detected in a previous study and was identified as 3-oxo-butanoic acid (Liu et al., 2020). However, the only commercially available isomer was 2-oxo-butanoic acid. The MS^2 was comparable to the detected formula but the compound showed a slightly different retention time (difference of ~ 0.5 min). 2-oxo-butanoic acid is not matching, but a butanoic acid with the oxo group at position 3 or 4 is likely (CL = 3, SI2.18). The derivatised mass of $C_4H_6O_3$ was also detected during ozonation of phenols and acetylacetone (Table 2 and Fig. SI2.18d). For the phenols no MS^2 was collected but for the ozonation product of acetylacetone it aligns very well (Fig. SI2.18a). Similar to pyruvic and glyoxylic acid, $C_4H_6O_3$ is formed with a secondary trend, also in line with phenols as model precursors (Figs. SI2.18b, d). At higher ozone doses the compounds remain stable due to an expected low k_{O_3} ($< 1 M^{-1}s^{-1}$ (Lee and von Gunten, 2012)).

$C_5H_6O_2$ (SI2.22). Three distinct isomers were detected for this formula. The structure of 4-oxo-2-pentenal is suggested as one of the isomers in analogy to 2-butene-1,4-dial (CL = 1, SI2.22). Methylphenol a potential precursor was not part of the present study and thus no comparison to product formation from model precursors is available. Fig. SI2.22b shows the formation trend, and Fig. SI2.22a a MS^2 comparison to synthesised 4-oxo-2-pentenal. The areas in samples in presence of an $\bullet OH$ scavenger are smaller than in absence. This is comparable to $C_4H_4O_2$, indicating that reactions with $\bullet OH$ are taking part in their formation in analogy to the formation of 2-butene-1,4-dial (Fig. SI2.22c, Prasse et al., 2018). This compound is removed to similar levels after sand-filtration compared to the samples prior to ozonation (Fig. SI8, SI1).

$C_5H_6O_3$ (SI2.23–24). Two distinct isomers were detected with this molecular formula (SI2.23, single derivatised and SI2.24, twice derivatised). 4-oxo-pentenoic acid was confirmed with a standard (SI2.23, CL = 1). Replicate measurements are quite scattered, indicating instability of the derivatised compound which is in line with observations for other ketoacids in this study (i.e. glyoxylic acid, Fig. S10, SI1). This compound is a primary-type product during ozonation. The compound shows lower areas in presence of an $\bullet OH$ scavenger (Fig. SI2.23c) indicating a higher abatement due to reactions with ozone (Section 3.2.2). An α, β -unsaturated ketoacid product is in line with past observations/suggestions of substituted phenols, where ring-opening products were hypothesised to be further oxidised to such unsaturated ketoacids (Tentscher et al., 2018). Furthermore, this compound is an analogue to $C_4H_4O_3$ formed from phenol which is mechanistically outlined in Fig. 4.

The second compound with the formula $C_5H_6O_3$ is derivatised twice (SI2.24, CL = 3). The dicarbonyl compound is formed with a secondary trend remaining stable at higher ozone doses, indicating no further reaction or a steady-state. Several candidates exist: an isomer of methyl furandione, a furancarboxaldehyde or a compound with three $C=O$ functional groups (SI2.24), which would be similar to $C_3H_2O_3$ (SI2.5). However, 3-methyl-tetrahydro-2,4-furandione (SI1) was only single-derivatised, which is clear as the other oxygens are located in an ester functional group and 2,3,4-pentanetrione and furancarboxaldehyde were not commercially available. These types of compounds are an example for a group of compounds (including $C_3H_2O_2$ (SI2.4) and $C_3H_2O_3$ (SI2.5)) which have not been reported as ozonation byproducts and need further investigations.

$C_5H_8O_2$ (SI2.26). This molecular formula is formed during ozonation of lake water and wastewater with a smaller slope. Interestingly, its peak area is higher in bench-scale- compared to plant-ozonated wastewater and in the presence of $\bullet OH$ (Fig. SI2.26c). In any water type, the peak area continuously increases and slightly levels off at high ozone doses, which is rationalised by the low expected reactivity with both ozone and $\bullet OH$. 2,3-pentanedione or 2- or 4-oxopentanal are likely candidates (CL

= 3, SI2.26), for this molecular formula (glutaraldehyde could be excluded (Manasfi et al., 2023)). Many structural isomers exist for this C_5 -formula, for example, acetylacetone, hydroxypentenals, or several methyl furanones. Acetylacetone ($k_{O_3} = 5 \cdot 10^5 M^{-1}s^{-1}$, Houska et al., 2021) and furanones (k_{O_3} ranging from 10^4 – $10^5 M^{-1}s^{-1}$, Zoumpoulis et al., 2021) can be excluded since they would not build up due to a high ozone reactivity. The standard 2,3-pentanedione matched with the retention time, however, the MS^2 spectra is ambiguous and therefore CL = 3 was assigned (Fig. SI2.26a). 2,3-pentanedione (Fig. SI2.26b) and 2,3-butanedione (Fig. SI1, SI1) show very similar formation trends upon ozonation, a further indication that they might be related compounds.

$C_5H_8O_3$ (SI2.27). This molecular formula has been reported and was identified as 3-methyl-2-oxo-butanoic acid (Liu et al., 2020). The derivatised mass of $C_5H_8O_3$ was also detected during ozonation of 4-ethylphenol (Table 2, Fig. SI2.27d) but at such a low intensity, that no MS^2 could be recorded.

Further compounds are compiled in SI2. They contain carbonyl groups for which only a limited selection of carbonyl compounds is feasible and (i) no commercial standards were available (SI2.2–4,10,21), (ii) a standard was purchased but did not match (SI2.11,20,25), (iii) information was gathered but no conclusive results were obtained (SI2.19) or (iv) isomers of carbonyl compounds that were discussed (SI2.13–15,30).

3.4.2. Nitrogenous carbonyl compounds

In total, 57 formulas containing at least one N atom were formed upon ozonation of the different water types. The formulas are found across a wide range of O:C and H:C ratios (Fig. S6b, SI1), indicating that in analogy to the carbonous carbonyl compounds, many structurally different types of nitrogenous compounds may be formed. Table 3 shows a selection of nitrogenous carbonyl compounds detected in this study (for all carbonyl compounds refer to SI3).

Typical *N*-DBPs formed during oxidation include haloacetonitriles, haloacetamides and halonitromethanes (Bond et al., 2011). Analogues which may be ozonation-induced could therefore include oxoacetonitriles, oxonitromethanes or oxoacetamides.

Cyanoformaldehyde (C_2HNO) would be the simplest oxoacetonitrile, which was reported at a very low yield during ozonation of drinking water (Richardson et al., 1999). In this study, also a peak at the corresponding mass was observed and the MS^1 fits to the number of N, however, no MS^2 spectrum was obtained and no standard was available. Potential candidates with a higher number of C such as oxopropanenitrile (C_3H_3NO) or cyanoacetaldehyde (C_4H_5NO) were not detected.

Nitroformaldehyde ($CHNO_3$), nitroacetaldehyde ($C_2H_3NO_3$) or e.g. nitropropanal ($C_3H_5NO_3$) could be expected as oxonitromethanes but none of the formulas was detected. However, the C_4 and C_5 analogues were detected. For $C_4H_7NO_3$ the suspect 4-nitro-2-butanone (other isomers were not available) was tested, but did not lead to a match with the MS^2 data. $C_4H_7NO_3$ is formed as a secondary product and it decreases at higher ozone doses (in the absence of an $\bullet OH$ scavenger), which suggests further reactivity with $\bullet OH$ (Section 3.2.2 and Fig. SI2.35b). Furthermore, the compound might be related to the *N*-containing carbonyls discussed below, which could indicate a hydroxy-oxo-butanamide. However, more data is needed for a conclusive assignment.

For oxoacetamides, three *N*-containing carbonyl compounds follow a pattern with increasing CH_2 : $C_2H_3NO_2$, $C_3H_5NO_2$ and $C_4H_7NO_2$. All of them show a neutral loss of m/z 45 (45.0207 ± 15 ppm, 45.0215 and 45.0215 (both $\pm < 5$ ppm), respectively), which likely corresponds to CH_3NO (monoisotopic mass of 45.0215). In a previous study, CH_2NO was proposed as neutral loss during negative ionisation to rationalise hydroxyphenylacetamide, which suggests that such neutral losses might be common (Gulde et al., 2021). The applied instrument had a measurement limit of 50 Da, thus, no lower fragments can be observed. No typical loss of 17.0270, 29.9980 or 45.9930 was observed and therefore amines, NO or nitro functional groups can be excluded (Ma et al., 2014). Control experiments demonstrated, that $C=O$ in an acetamide or

Table 3

Selected nitrogenous carbonyl compounds (for complete list refer to SI3) detected in different water types. The formulas are sorted by increasing number of carbons from top to bottom.

formula of carbonyl compound	(tentative) name	confidence level	formation trend	number of derivatised sites	accurate mass of derivatised form	retention time (min)	Lake Greifensee	Lac de Bret	WWTP Glarnerland	WWTP Neugut	WWTP Werdhölzli	Werdhölzli WW plant	SRFA
C ₂ H ₃ NO ₂	oxo-acetamide	2	(iv)	1	241.0518	13.1	(✓)	(✓)	✓	✓	✓	✓	✓
C ₃ H ₂ N ₂ O ₃	2-oxo-propanamide	3	(iv)	1	282.0425	14.0	✓	(✓)	✓	✓	✓	✓	✓
C ₃ H ₅ NO ₂		1	(iv)	1	255.0674	13.2	✓	(✓)	✓	✓	✓	✓	✓
C ₄ H ₃ N ₃ O ₂	oxo-butanamide	3	(v)	2	461.0941	17.0			✓	✓	✓	(✓)	
C ₄ H ₃ N ₃ O ₃		3	(v)	1	309.0531	14.5			✓	✓	✓	(✓)	
C ₄ H ₄ N ₂ O ₃		3	(iv)	1	296.0584	15.3	✓		✓	✓	✓	(✓)	✓
C ₄ H ₅ NO ₂		3	(v)	1	267.0677	12.6					✓	✓	
C ₄ H ₅ NO ₄	oxo-butanamide	3	(iv)	1	299.0575	13.6			✓	✓	✓	(✓)	(✓)
C ₄ H ₆ N ₂ O		3	(v)	1	266.0837	16.2	(✓)	(✓)	✓	(✓)	✓	✓	(✓)
C ₄ H ₇ NO ₂		3	(i)	1	269.0838	13.8	(✓)	(✓)	✓	✓	✓	(✓)	(✓)
C ₄ H ₇ NO ₃		3	(v)	1	285.0786	14.6	✓	✓	(✓)	✓	✓	✓	(✓)
C ₅ H ₉ NO ₄	oxo-butanamide	3	(iv)	2	483.1244	16.8			✓	✓	✓	(✓)	
C ₅ H ₆ N ₂ O ₃		3	(iv)	1	310.0733	14.5	✓	(✓)	✓	✓	✓	✓	✓
C ₅ H ₇ NO ₄		3	(iv)	1	313.0731	13.8	(✓)	(✓)	✓	✓	✓	✓	(✓)
C ₅ H ₉ NO ₃		3	(v)	1	299.0940	15.2			✓	✓	✓	✓	

Ticks in brackets: a peak with this mass and retention time was detected but not detected by our workflow (peak area between 1-2 in ozonated sample vs. non-ozonated sample or MS² was too weak or absent). Formation trends: (i) primary with constant maximum, (ii) primary with further abatement, (iii) products with primary trend but less efficient formation, (iv) secondary product with constant maximum and (v) secondary product with further abatement.

formamide and also a nitroso group cannot be derivatised (Table S1, SI1) and thus such structures can be excluded. Nevertheless, C₃H₅NO₂ was identified as 2-oxo-propanamide by a standard (CL = 1, SI2.33) which was unexpected since 3-oxo-butanamide (acetoacetamide) could not be derivatised. However, the latter likely exists in its enol form, which disables derivatisation. If this information is applied to the C₂ and C₄ analogues, the oxo group is either located at the α- or γ-C of the amide leading to 2-oxo-acetamide (CL = 2, no standard available) for C₂H₃NO₂ and 2- or 4-oxo-butanamide for C₄H₇NO₂ (CL = 3, no standard available). During ozonation, 2-oxo-propanamide is formed with a secondary trend levelling off at higher ozone doses (Fig. SI2.33b). The ozone and •OH reactivity of this compound is low (von Sonntag and von Gunten,

2012). Comparison of DOC concentration-normalised areas amongst the different water types indicates a much higher abundance in wastewater and its presence is not significantly influenced by the presence of an •OH scavenger (Fig. SI2.33c). The latter indicates similar extents of formation by ozone and •OH pathways (Section 3.2.2). The C₂ and C₄ analogues show very similar behaviour except that C₄H₇NO₂ is more efficiently formed (SI2.31 and SI2.34). A hypothetical formation mechanism for C₂H₃NO₂ and C₃H₅NO₂ is outlined in Figs. 5 and 6, respectively. Fig. 5 shows direct ozone attacks on the nucleobases uracil and thymine (1). After the formation of an ozonide, different intermediates (2, 3) are formed, which may release C₂H₃NO₂ and C₃H₅NO₂, respectively (4) (Flyunt et al., 2002). The secondary trends

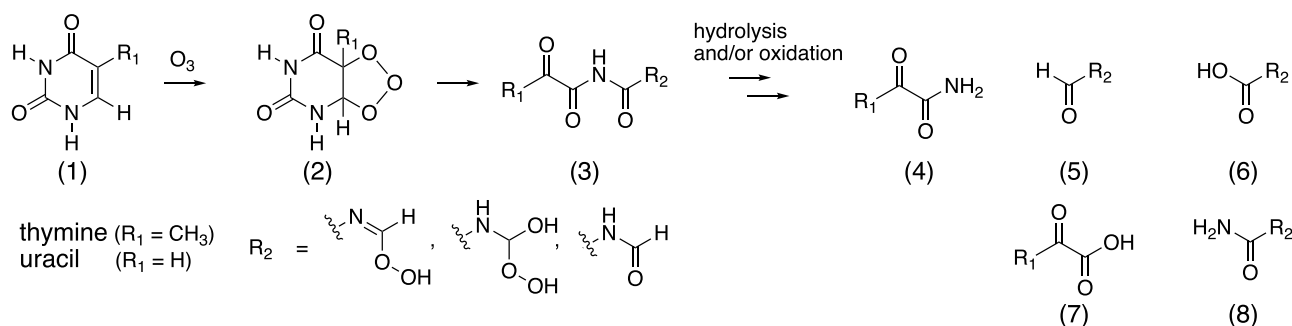


Fig. 5. Proposed mechanisms for the reactions of the nucleobases thymine (R₁=CH₃) and uracil (R₁=H) with ozone (1) via intermediates (2)-(3) to various carbonyl-containing byproducts (4)-(8). (4) R₁=H: C₂H₃NO₂; R₁=CH₃: C₃H₅NO₂.

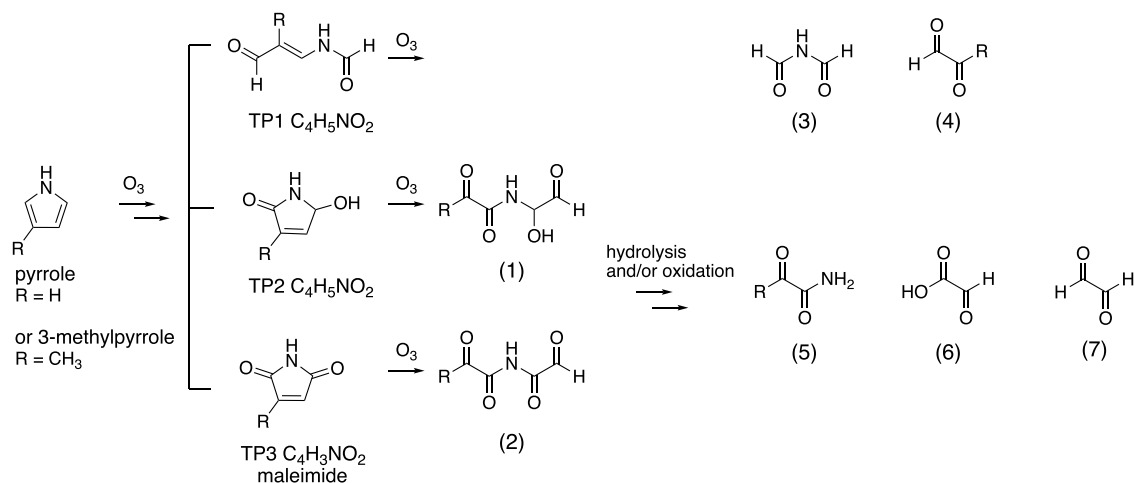


Fig. 6. Proposed mechanisms for the reactions of pyrrole with ozone. Transformation products 1–3 (TP1–3 (Tekle-Röttering et al., 2020)) further react via intermediates (1) and (2) to various carbonyl-containing byproducts (3)–(7).

align with this information, where $C_2H_3NO_2$ is only formed at a higher ozone dose compared to $C_3H_5NO_2$. This can be explained by the lower second-order rate constant of uracil ($R_1 = H$, $k_{O_3} \sim 10^2 M^{-1}s^{-1}$) compared to thymine ($R_1 = CH_3$, $k_{O_3} \sim 10^4 M^{-1}s^{-1}$) (Theruvathu et al., 2001).

A previous study proposed that $\bullet OH$ reactions may lead to a cleavage of the C–N bond with a release of amides (Gulde et al., 2021). The formation of $C_2H_3NO_2$ ($R_1 = H$) and $C_3H_5NO_2$ ($R_1 = CH_3$) (4) did not differ in the presence and absence of a $\bullet OH$ scavenger (SI2.31–33) which indicates, that these products are formed from precursors via both ozone and $\bullet OH$ pathways (Section 3.2.2). However, the mechanism leading to products (4) and (5) is currently unclear.

Moreover, the carbon atoms with adjacent electron-withdrawing carbonyl and amide groups are electron-deficient and thus susceptible to nucleophilic attack. Consequently, a nucleophilic attack by water (or OH^-) seems possible leading to acids (6,7) and amides (8).

Fig. 6 shows the ozonation mechanism of pyrrole ($R=H$). According to Tekle-Röttering et al., 2020, three transient ozonation byproducts are discussed (TP1 or TP2 = $C_4H_5NO_2$ and TP3 = $C_4H_3NO_2$ (maleimide)). TP1 contains a double bond, which might directly react to carbonyl-containing products $C_2H_3NO_2$ (3) and $C_2H_2O_2$ (glyoxal, 4). TP2 and maleimide contain a double bond which may react with ozone to ring-opened products (1) or (2), that may further be cleaved to $C_2H_3NO_2$ (5) and other carbonyl-containing products (6) and (7). If a similar structure containing an additional methyl group exists (i.e. 3-methylpyrrole with $R = CH_3$), analogues with a higher number of C-atoms are possible.

Besides the molecular formula proposed as oxoacetamide (5), also molecular formulas coinciding with the transient products TP1, TP2 and (1) were detected (Fig. 6). Their formation trends upon ozonation are shown in Fig. S17 (SI1). With all this evidence taken together, such structures are likely formed.

$C_5H_6N_2O_3$ and $C_3H_2N_2O_3$ (SI2.32 and SI2.36) are two additional N-containing carbonyl compounds which were frequently formed. Both show a clear primary formation pattern and a maximum at higher specific ozone doses ($> 1 \text{ mgO}_3/\text{mgC}$). Nevertheless, there is not sufficient information to assign clear structures to these formulas.

4. Conclusions

Carbonyl compounds are a major class of ozonation-induced oxidation or disinfection byproducts from water matrix components such as dissolved organic matter. In this study the following aspects are covered: (1) formation of carbonyl compounds during ozonation for a wide range of different water types, (2) link between carbonyl compounds and their

precursors by ozonation of model compounds and (3) identification of a set of carbonyl compounds by a MS-based non-target screening method combined with kinetic and mechanistic information. The main findings are:

- 178 diverse carbonyl compounds were formed during ozonation of different water types (2 lake waters, 3 wastewaters, 1 full-scale wastewater treatment plant, Suwannee River fulvic acid-containing water).
- The carbonyl compounds can be classified in five different formation trends as a function of specific ozone doses.
- Eight carbonyl compounds formed during ozonation could be identified with confidence level 1 and seven with confidence level 2. Some carbonyl compounds such as hydroxyacetone, unsaturated dialdehydes and ketoacids, potentially trialdehydes/ketones and the N-containing 2-oxo-propanamide are reported for the first time in real ozonated water samples.
- An overlapping subset (20%) of carbonyl compounds is detected during ozonation of different water types. Sample treatment conditions (presence of a $\bullet OH$ scavenger, pH control) have a minor influence on the detectability of carbonyl compounds during ozonation.
- For given specific ozone doses, the concentration level of a carbonyl compound in a particular water depends on the DOC concentration.
- Ozonated municipal wastewater effluents show a significantly higher fraction of N-containing carbonyl compounds (30%) compared to lake water (17%).

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.2022.119484.

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