



Analysis of ultrasonic pre-treatment for the ozonation of humic acids

Pello Alfonso-Muniozguren^{a,b}, Cristian Ferreiro^b, Elodie Richard^{a,c}, Madeleine Bussemaker^a, José Ignacio Lombrana^{b,*}, Judy Lee^{a,*}

^a Chemical and Process Engineering, University of Surrey, Guildford GU27XH, United Kingdom

^b Department of Chemical Engineering, Faculty of Science and Technology, University of the Basque Country UPV/EHU, 48940 Leioa, Spain

^c Department of Chemistry, IUT Besançon-Vesoul, University of Franche-Comté, 25000 Besançon, France

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ABSTRACT

This paper presents an intensification study of an ozonation process through an ultrasonic pre-treatment for the elimination of humic substances in water and thus, improve the quality of water treatment systems for human consumption. Humic acids were used as representative of natural organic matter in real waters which present low biodegradability and a high potential for trihalomethane formation. Ultrasonic frequency (98 kHz, 300 kHz and 1 MHz), power (10–40 W) and sonicated volume (150–400 mL) was varied to assess the efficiency of the ultrasonic pre-treatment in the subsequent ozonation process. A direct link between hydroxyl radical (HO[•]) formation and fluorescence reduction was observed during sonication pre-treatment, peaking at 300 kHz and maximum power density. Ultrasound, however, did not reduce total organic carbon (TOC). Injected ozone (O₃) dose and reaction time were also evaluated during the ozonation treatment. With 300 kHz and 40 W ultrasonic pre-treatment and the subsequent ozonation step (7.4 mg O₃/L_{gas}), TOC was reduced from 21 mg/L to 13.5 mg/L (36% reduction). HO[•] attack seems to be the main degradation mechanism during ozonation. A strong reduction in colour (85%) and SUVA₂₅₄ (70%) was also measured. Moreover, changes in the chemical structure of the macromolecule were observed that led to the formation of oxidation by-products of lower molecular weight.

1. Introduction

Humic acids (HAs) are naturally occurring high molecular weight compounds easily found in natural water bodies. They are the main component of organic matter in natural waters and responsible for colour (yellow to black). These compounds are difficult to mineralise completely as HAs tend to be refractory and possess low biodegradability. Additionally, HAs can act as trihalomethane precursors [1]. The effects of ultrasonication on HAs have been studied in the literature, showing a potential for humic acid (HA) degradation (change in molecular structure), as well as an increase in biodegradability.

At low frequencies (20 kHz), changes in UV absorption of HA samples have been reported [2]. According to the authors, sonication could have induced alterations in the molecular structure of HAs. With an initial TOC concentration of 18.6 mg/L (equivalent to 50 mg HA/L) and 50 mg NaOCl/L, a TOC removal of 26.5% was reported after 120 min of treatment at 20 kHz [3]. Similar reactions have been measured at higher frequencies (200 kHz), e.g. a change in UV absorption and a reduction in the molecular weight of HAs [4]. Chen et al. [5] employed two different

ultrasonic reactors for the treatment of synthetic HA solutions (22.5 mg/L initial TOC): a 20 kHz horn and a 354 kHz transducer. 354 kHz reactor performed better in the reduction of TOC, achieving a TOC of 20.4 mg/L after 4 h of treatment. No TOC removal was observed with 20 kHz. The authors mentioned that even though TOC was not significantly reduced, the reduction in colour, as well as in SUVA₂₅₄ (specific ultraviolet absorbance at 254 nm where the aromatic nature of the solution is normalised over the total organic load [6]) would be translated into a change in molecular structure (destruction of aromatic rings, conjugated double bonds, etc.). Although different applied frequencies and power densities have been reported, variations in sonication equipment and reactor design makes it difficult to compare different studies to obtain a reliable conclusion on the best parameters for HA removal with ultrasound. Considering chemical and physical effects produced by ultrasound vary significantly depending on the applied frequency, power and sonication system [7–9], there is a need for a thorough study on the influence of these parameters on the degradation of HAs.

Ozone (O₃) has also been employed for the treatment of HAs. With an initial TOC concentration of 100 mg/L and an O₃ saturated solution at

* Corresponding authors.

E-mail addresses: ji.lombrana@ehu.eus (J.I. Lombrana), j.y.lee@surrey.ac.uk (J. Lee).

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0.46 mol/m³, TOC removal increased from 50% to 80% when treatment time was increased from 30 min to 5 h [10]. A complete mineralisation was difficult to reach considering refractory compounds were formed during ozonation. Besides TOC, O₃ can significantly reduce the molecular size distribution, colour₄₃₆ and UV₂₅₄ of HAs [11,12], as well as increase its biodegradability [11,13]. The authors, however, concluded that ozonation alone is insufficient to treat humic water [13].

The combination of O₃ and ultrasound to effectively treat HA has also been studied in the literature. Olson and Barbier [14] coupled ultrasound (20 kHz horn) and O₃ to treat a purified fulvic acid fraction of synthetic HA solution (10 mg/L initial TOC). Ultrasound alone had no effect on the absorbance of fulvic acid at 200 and 340 nm, whereas combined with O₃ the rate of decolouration (230 nm) increased. In regards to TOC, ultrasound alone did not mineralise carbon. When O₃ was applied and during the first 10 min of treatment, the same oxidation rate of TOC was observed between O₃ alone and the combined setup. Increasing treatment time in the coupled system induced a significant enhancement in TOC removal. According to the authors, pyrolysis mechanisms would explain the increase in O₃ decomposition rate due to ultrasound. Weavers et al. [15,16] stated that when O₃ is combined with ultrasound, an increase in O₃ mass transfer is observed due to the turbulence produced by acoustic streaming. Stepniak et al. [17] also used ultrasound and O₃ to treat synthetic HA solutions of 10, 15 and 20 mg HA/L. A 24 kHz ultrasonic horn with a maximum effective power of 300 W was used for sonication experiments. Two different configurations were employed when combining O₃ and ultrasound. In the first configuration, O₃ was first applied to the HA solution (3 mg O₃/L for 3 min) and sonicated later on in a subsequent step (10 min) reaching a TOC removal of 25.3%. The second configuration combined O₃ and ultrasound simultaneously for 10 min. The study reported no synergy when ultrasound and O₃ were applied simultaneously (second configuration), showing a slight increase in TOC removal to 28.8% with the coupled system. Chemical modification of aromatic structures of HAs were reported in the combined configurations. An increase in treatment time and increasing injected O₃ dose from 1 to 3 and 5 mg/L led to a higher removal of TOC for the coupled system.

Considering the above, the potential impact from the pre-treatment using ultrasound in a subsequent treatment process (i.e. ozonation) has not been investigated in the literature. Therefore, this research article presents a thorough study on the effect of an ultrasonic pre-treatment in a subsequent ozonation step for the treatment of HAs in waters intended for human consumption.

2. Materials and methods

2.1. Chemicals

HA (CASRN: 1415-93-6, Sigma-Aldrich, technical), sulfuric acid (H₂SO₄, Sigma-Aldrich, 98%), sodium hydroxide (NaOH, Panreac, 0.25 M), potassium iodide (KI, Panreac, 99%), sodium phosphate monobasic (H₂O₄PNa·H₂O, Fisher, 98%) and sodium phosphate dibasic heptahydrate (HNa₂O₄P·H₂O, Fisher, 99%) were used as received. Deionised water was supplied by a Milli-Q® water purification unit supplied by Merck.

2.2. Experimental procedure

HA sample preparation was carried out as follows: 100 mg HA were mixed with 2 L deionised water (50 mg HA/L) and dissolved by increasing the pH to 11 with 0.25 M NaOH. The dissolution was then stirred for 30 min at 400 rpm. Prior to sonication experiments, pH was reduced to 7 with 0.1 M H₂SO₄. Elemental analysis (CHONS), fluorescence, ¹³C NMR, FTIR and HPSEC spectrum of the employed HAs are shown in [supplementary information \(Fig. S1\)](#).

Sonication pre-treatment experiments (Fig. 1a) were carried out in a jacketed cylindrical glass vessel (15 cm height and 6.7 cm inner

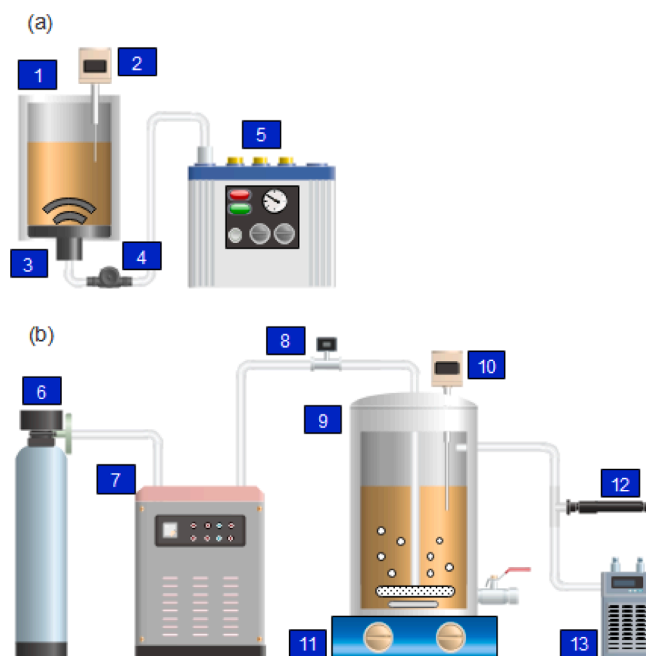


Fig. 1. Schematic of the experimental setup. (a) Pre-sonication setup; (1) Sonication vessel, (2) pH meter and thermometer, (3) Plate transducer, (4) Fuse, (5) Power amplifier. (b) Ozonation setup, (6) O₂ bottle, (7) O₃ generator, (8) Flow metre, (9) O₃ reactor, (10) pH meter and thermometer, (11) Magnetic stirrer, (12) O₃ destroyer, (13) O₃ analyser.

diameter). The ultrasonic transducer (Honda Electronics Co. LTD) was placed at the bottom of the vessel and powered by a power amplifier (T&C Power Conversion AG1006). Three different frequencies (98 kHz, 300 kHz and 1 MHz) at four different applied powers (10, 20, 30 and 40 W) were employed, varying sample volume from 150 mL to 400 mL. HA samples (50 mg HA/L) were sonicated for 10, 20 and 30 min. pH and temperature were continuously monitored (model GLP 22 from Crison) before and after pre-sonication experiments. When needed, pH was adjusted to 7 prior to ozonation.

Sonicated effluent was further treated with O₃ (Fig. 1b) in a 2 L cylindrical glass reactor previously described [18]. Two O₃ diffusers and a magnetic stirrer were placed at the bottom of the reactor, maintaining a constant injection gas flowrate (4.76 L/min) and mixing speed (60 rpm). O₃ was generated in-situ by the Triogen Lab2B generator from extra pure oxygen. 1 L pre-sonicated HA solution was ozonated for 30 and 120 min with O₃ injection at four different dosages: 5.4, 7.4, 11.3 and 19.7 mg O₃/L_{gas}. Dissolved O₃ (Rosemount Analytical model 499AOZ-54 probe) and exhaust O₃ (MT 964C ozone analyser) were continuously monitored, as well as dissolved O₂ (Rosemount Analytical Solu Comp II recorder), pH and temperature (Rosemount Analytical model 399-09-62 probe). Exhaust O₃ was destroyed by a Zonosistem thermocatalytic ozone destructor. All experiments were run at least in duplicates.

2.3. Analytical methods

Carbon, hydrogen, nitrogen and sulphur content present in the HA were determined by Euro EA Elemental Analyzer (CHNS). 10 mg of HA was completely oxidised by combustion with oxygen at a temperature of 1020 °C and combustion products identified with a thermal conductivity detector. Fourier-transform infrared spectroscopy (FTIR) was conducted with a Jasco 4200 model to determine FTIR values in infrared medium (4000–400 cm⁻¹) using KBr pellets for solid sample preparation. Pellet preparation was carried out by mixing 1.5 mg HA with 300 mg KBr. The mixture was then subjected to high vacuum compression until a 10 mm diameter and 1.5 mm thick pellet was obtained. Liquid sample analyses were carried out by evaporating 240 µL of sample at 80 °C and

atmospheric pressure in a 13×2 mm ZnSe tablet (Pika Technologies) placed inside an oven. ^{13}C NMR spectra were measured with a Bruker Avance III 400 MHz NMR spectrometer configured with the following characteristics: resonance frequency 100.62 MHz; $\pi/2$ pulse length 13.1 μs ; acquisition time 0.15 s; relaxation delay 1.5 s, $^1\text{J}_{\text{H-C}} = 7.5$ Hz and 120 scans. The pKa (acid dissociation constant) of the HA sample was calculated using UV–Visible spectrophotometry (PerkinElmer Lamda 10 UV/Vis spectrophotometer) and according to the method described by Reijenga et al. [19]. The determination of the E_3/E_5 ratio (ratio between UV absorbance at 350 and 550 nm and related to the molecular weight of humic substances) was carried out by dissolving 2 mg of HA in 10 mL of 0.05 M NaHCO_3 and a subsequent absorbance measurement by Perkin Elmer Lamda 10 UV/Vis spectrophotometer at wavelengths of 350 and 550 nm [20].

A Water 2695 HPSEC system with an Agilent ZORBAX GF-250 column (4.6×250 mm, 4 μm) was used for HPSEC analyses. A phosphate buffer (100% at pH 7) was injected at a flowrate of 1 mL/min with an ionic strength of 0.2 M and an injection volume of 20 μL . The Waters 2487 absorbance detector was used at a wavelength of 254 nm. The HPSEC system was calibrated with protein standards (Sigma-Aldrich) of known molecular weight between 14 and 600 kDa. A semi-exponential curve was obtained and used ($\text{MW} = 3 \times 10^8 \times t_{\text{R}}^{-9.691}$ with an $R^2 = 0.999$ for $\text{MW} > 47$ kDa and $\text{MW} = 21233 \times t_{\text{R}}^2 - 171755 \times t_{\text{R}} + 343352$ with an $R^2 = 1$ for $\text{MW} \leq 47$ kDa) for the determination of molecular weights at different retention times. Shimadzu TOC-VCSH Analyser was used for TOC measurements. Perkin Elmer LS-50B luminescence spectrophotometer (pre-sonication experiments) and Jasco FP-8200 fluorimeter (ozonation experiments) were used for fluorescence analyses, running a synchronous spectrum with an excitation spectra between 250 and 550 nm, a displacement of 20 nm and a scanning speed of 100 nm/min. Colour (PerkinElmer Lamda 10 UV/Vis spectrophotometer at 455 nm [21]) and SUVA_{254} (PerkinElmer Lamda 10 UV/Vis spectrophotometer at 254 nm) were also monitored. The concentration of I_3^- (proportional to the concentration of oxidising agents such as hydroxyl radicals) was measured following the KI dosimetry method [22,23].

3. Results and discussion

3.1. Ultrasonic pre-treatment

No significant TOC removal was measured after 30 min ultrasonic pre-treatment (300 kHz and 40 W). Therefore, impact of ultrasonic pre-treatment on the fluorescence spectra were investigated for any change at molecular level.

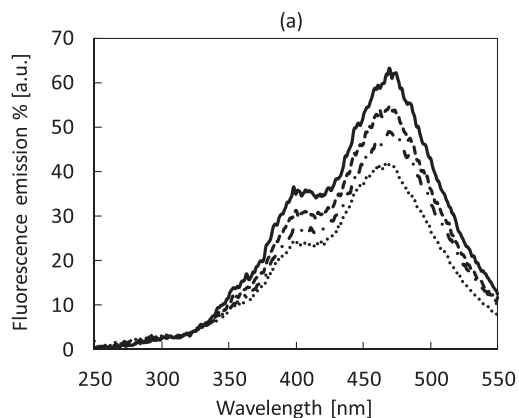


Fig. 2. Effect of ultrasound (40 W) on fluorescence emission of 50 mg HA/L samples. (a) Fluorescence emission percentage after 30 min and 400 mL sample. Initial sample with no sonication (—), 98 kHz (---), 1 MHz (- · -) and 300 kHz (.....). (b) Fluorescence emission intensity reduction percentage at 470 nm as a function of I_3^- concentration at 10, 20 and 30 min. 98 kHz (empty symbols), 300 kHz (black filled symbols), 1 MHz (grey filled symbols) and 150 mL (\circ), 200 mL (Δ), 300 mL (\diamond), 400 mL (\square) samples.

3.1.1. Fluorescence spectra and degradation mechanisms

Fluorescence emission intensity was monitored during ultrasonic pre-treatment in order to evaluate the impact of different frequencies and power densities on the fluorescence emission intensity band. Changes to the fluorescence emission indicates a change in the molecular structure of HAs [24], although this was not translated into carbon mineralisation (TOC removal).

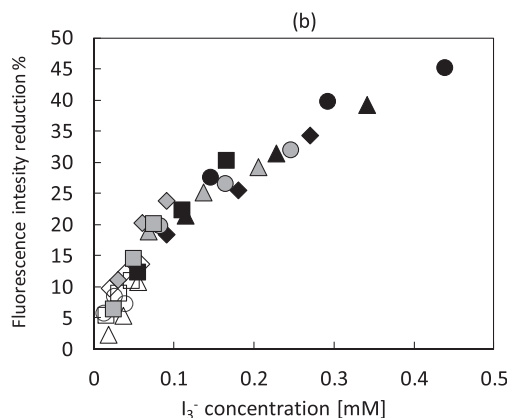
Fig. 2a shows the reduction in fluorescence intensity after 30 min of treatment at an applied power of 40 W for 98 kHz, 300 kHz and 1 MHz in 400 mL samples. Fluorescence intensity at λ_{max} was reduced the most at 300 kHz (31%), followed by 1 MHz (20%) and 98 kHz (11%). At 300 kHz, the reduction in fluorescence intensity was proportional to the applied power density (Fig. S2a). Reducing sample volume at a given applied power (increase in power density) also leads to a higher production of HO^\bullet [25] and has a similar effect on the fluorescence intensity reduction as decreasing applied power (Fig. S2 b). It is well known that ultrasound can produce HO^\bullet through the dissociation of water vapour when a cavitating bubble collapses [26,27], also leading to the formation of localised microjets when bubbles collapse asymmetrically near a surface [28,29]. These chemical (HO^\bullet) and physical (shear forces produced by microjets) processes are the principal mechanisms taking part in the degradation of compounds when ultrasound is applied. At low frequencies (98 kHz) physical effects are predominant due to a stronger collapse of the bubbles, while the highest HO^\bullet production is observed at medium–high frequencies (300 kHz) [26].

To evaluate whether the HO^\bullet production is the mechanism behind the reduction in fluorescence emission intensity of HAs, the concentration of HO^\bullet was evaluated in terms of concentration of I_3^- formed for each of the frequencies and different sample volumes. This was plotted against the fluorescence intensity reduction (Fig. 2b) to investigate its correlation that is independent of the applied frequency, increasing treatment time, applied power and sample volume. The strong correlation shown in Fig. 2b confirms that the reduction in fluorescence intensity could be mainly attributed to the attack of oxidising agents (HO^\bullet primarily) produced by ultrasound, and not to the mechanical (physical) effects.

3.2. Ozonation treatment

3.2.1. Effect of ozone injection dose on TOC

O_3 injection dose was varied (5.4, 11.3 and 19.7 $\text{mg O}_3/\text{L}_{\text{gas}}$) to maximise O_3 injection efficiency relative to TOC removal. Fig. 3 shows that increasing injected O_3 dose, TOC removal rate increased within the first ten minutes of the experiment. However, further increase in ozonation time up to 30 min, led to a similar TOC removal percentage for the



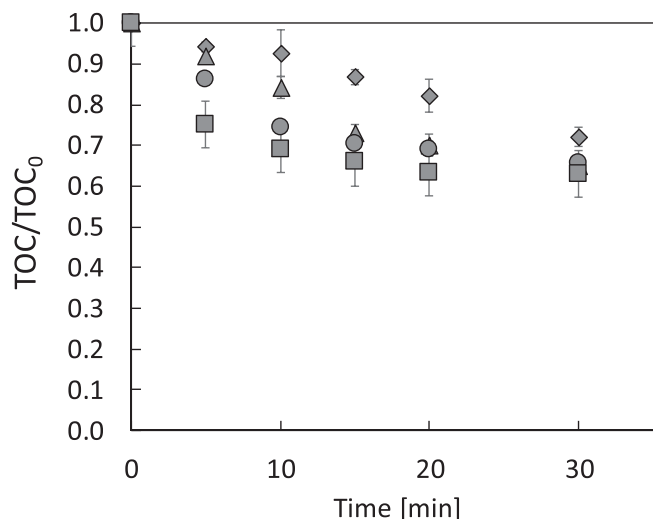


Fig. 3. TOC concentration as a function of treatment time for O_3 alone for different injected O_3 dosages of 5.4 mg O_3/L_{gas} (\blacklozenge), 7.4 mg O_3/L_{gas} (\blacktriangle), 11.3 mg O_3/L_{gas} (\circ), 19.7 mg O_3/L_{gas} (\square), 19.7 mg O_3/L_{gas} (\bullet).

three highest applied O_3 dosages ($\sim 36\%$ reduction) and 27.8% reduction was measured for the lowest applied O_3 dosage of 5.4 mg O_3/L_{gas} . The difference in TOC removal rates between the aforementioned O_3 dosages (mainly 7.4 mg O_3/L_{gas} and 11.3 mg O_3/L_{gas}) would come from the action of molecular O_3 , considering a similar concentration of HO^\bullet would be expected from the two injection dosages (Fig. S3). Increasing O_3 injection dose increased dissolved O_3 concentration (Fig. S4a) in the HA solution, leading to a higher TOC removal rate within the first minutes of the treatment. This is supported by a strong correlation between TOC removal and dissolved O_3 concentration (Fig. S4b).

Once O_3 was transferred to the aqueous phase, a decrease in TOC was measured during the first stages of degradation probably due to decarboxylation reactions that can strongly oxidise the humic structure [30]. However, given the nature of the HA used, after the first degradation stages, the rate of oxidation decreased. The appearance of degradation by-products such as acids and esters could be responsible for the reduction in the oxidation rate, which have a lower reactivity ($k = 0.0019 \text{ mg TOC}^{-1} \text{ s}^{-1}$) than the aromatic groups, alcohols and phenols ($k = 0.071 \text{ mg TOC}^{-1} \text{ s}^{-1}$) that initially predominate in the first oxidation states of the HA structure [31]. The identification of these by-products was carried out using the FTIR spectra in Fig. 8, where an increase in 1260 cm^{-1} and 1720 cm^{-1} bands was observed, indicative of the formation of carboxylic acids. In addition to these organic compounds that are formed during ozonation, there are other inorganic compounds that are difficult to remove, such as bromate ions. Therefore, the increase in the TOC elimination rate in the initial stages of the treatment could be also related to the characteristics of the HA at that specific time [32].

3.3. Ultrasonic pre-treatment and ozonation

3.3.1. Effect on TOC

With 30 min ultrasonic pre-treatment (300 kHz and 40 W) and a subsequent ozonation (Fig. 4), a similar behaviour in TOC removal was observed. It is important to highlight, however, that the TOC reduction rate between 7.4 mg O_3/L_{gas} and 11.3 mg O_3/L_{gas} seemed to be similar, contrary to non-sonicated values (Fig. 3). This could be related to the change in the molecular structure of HAs during sonication pre-treatment stated earlier, leading to a lower demand in O_3 (reduction from 11.3 mg O_3/L_{gas} to 7.4 mg O_3/L_{gas}) to obtain a similar TOC removal rate. Similarly, Ma and Lin [3] showed that ultrasound pre-treatment (combined with O_2) decreased the consumption of chlorine

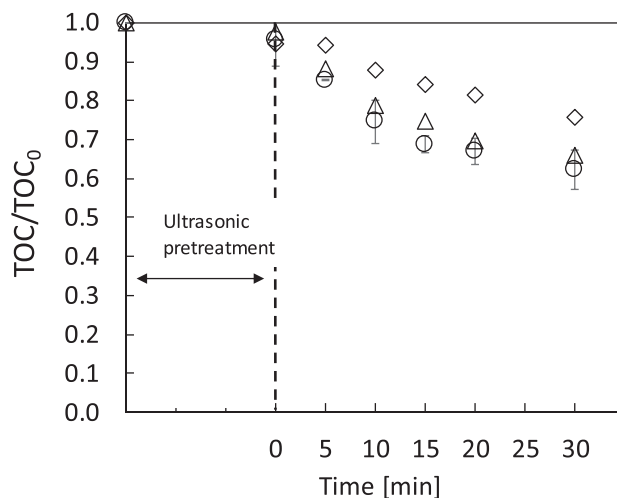


Fig. 4. TOC concentration as a function of treatment time for injected O_3 dosages of 5.4 mg O_3/L_{gas} (\diamond), 7.4 mg O_3/L_{gas} (Δ), 11.3 mg O_3/L_{gas} (\circ), 19.7 mg O_3/L_{gas} (\square). 30 min ultrasound (300 kHz and 40 W) pre-treatment applied 400 mL sample and 50 mg HA/L.

in a subsequent chlorination process. Ultrasonic pre-treatment, nonetheless, did not increase the final TOC removal of O_3 treatment for the two abovementioned applied O_3 dosages. As it happened with non-sonication experiments, the lowest TOC removal percentage (24.3%) was once again obtained with 5.4 mg O_3/L_{gas} . For a given O_3 treatment (mg O_3/L_{gas}), pre-sonication at different frequencies had no notable impact on the TOC reduction rate. (Fig. 5).

3.3.2. Effect on colour and $SUVA_{254}$

For both ozonation alone and pre-sonication/ozonation experiments, a significant reduction in colour was observed. However, 30 min of sonication (300 kHz and 40 W) showed no significant reduction in colour, compared to 85% colour removal with ozonation treatment (Fig. 6a). Therefore, the colour reduction can be attributed to the breaking of polymers of humic material in water and the reactivity of O_3 with chromophore groups [33]. Although the final colour removal percentage was similar for the three O_3 dosages used (5.4, 7.4 and 11.3 mg O_3/L_{gas}), a difference in colour removal rates between 5.4 and 7.4 mg O_3/L_{gas} was evident (e.g. compared to 7.4 mg O_3/L_{gas} , colour values

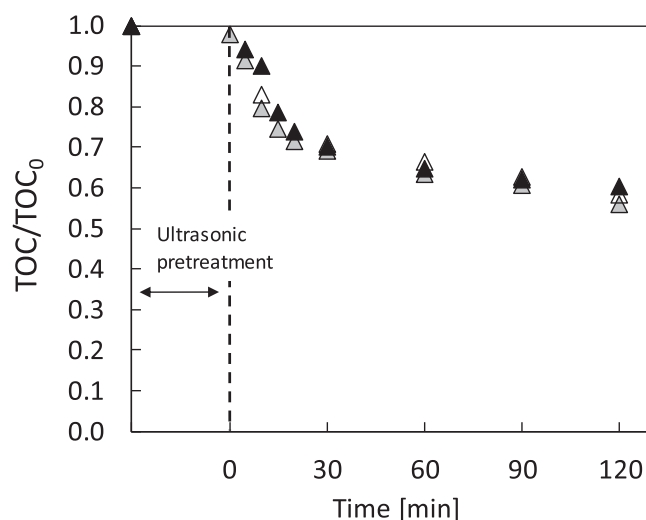


Fig. 5. TOC concentration as a function of treatment time for 30 min ultrasonic pre-treatment (40 W) and 120 min O_3 treatment (7.4 mg O_3/L). 98 kHz (\blacktriangle); 300 kHz (\blacktriangle); 1 MHz (Δ). 400 mL sample and 50 mg HA/L.

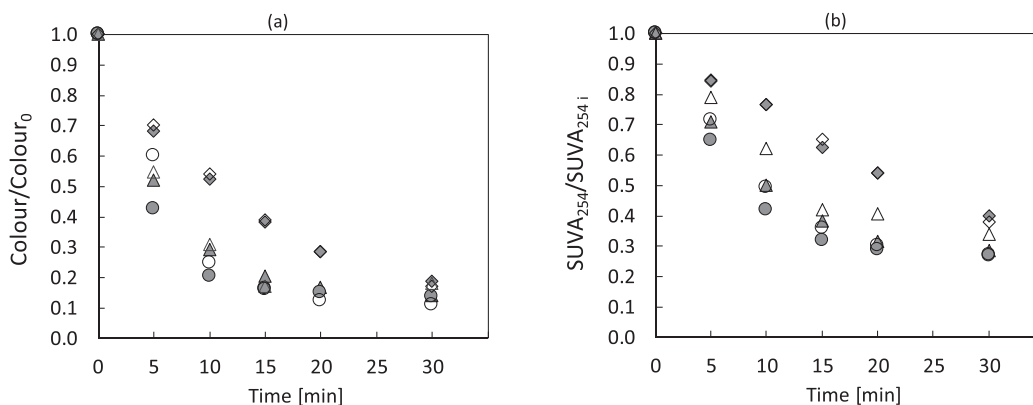


Fig. 6. HA degradation analysis through: (a) Colour as a function of time. (b) SUVA₂₅₄ as a function of time. 5.4 mg O₃/L_{gas} (◇), 7.4 mg O₃/L_{gas} (Δ) and 11.3 mg O₃/L_{gas} (○). O₃ alone (grey symbols), and 30 min ultrasound + O₃ (empty symbols). 300 kHz and 40 W for ultrasound experiments. 400 mL sample and 50 mg HA/L.

at 10 and 15 min were double for 5.4 mg O₃/L_{gas}).

Similarly, ultrasonic pre-treatment had negligible effect on the SUVA₂₅₄ (Fig. 6b) and a reduction of 70% was measured for the two highest O₃ dosages, reducing slightly the removal percentage to 62% with 5.4 mg O₃/L_{gas}. The difference during ozonation in SUVA₂₅₄ removal rates between 5.4 and 7.4 mg O₃/L_{gas} was also maintained in this case (e.g. SUVA₂₅₄ values at 10 and 15 min).

3.4. Degradation mechanisms

3.4.1. HO[•] yield

As indicated in Fig. 2b there is a strong correlation between HO[•] (quantified by measuring concentration of I₃⁻) and the degradation of HAs. Fig. S3 shows that the concentration of I₃⁻ achieved with O₃ (7.4 and 11.3 mg O₃/L_{gas} injected) is approximately 10 times higher than the

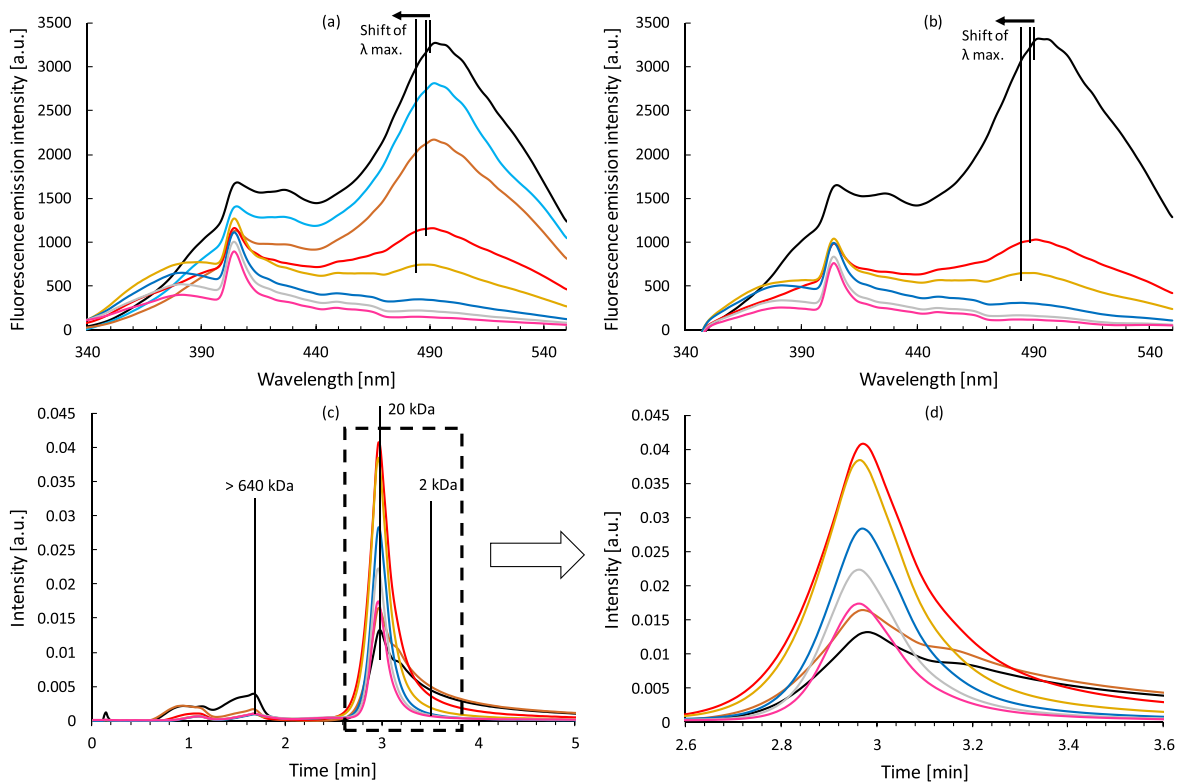


Fig. 7. HA degradation analysis through: (a) Fluorescence emission intensity US + O₃; (b) Fluorescence emission intensity O₃ alone; (c) HPSEC analysis US + O₃; (d) Zoomed in plot of (c) at 2.6–3.6 min time interval. (a, c, d) Initial sample (—), 10 min ultrasound (US, —), 30 min US (—), 30 min US + 5 min O₃ (—), 30 min US + 10 min O₃ (—), 30 min US + 15 min O₃ (—), 30 min US + 20 min O₃ (—) and 30 min US + 30 min O₃ (—); (b) Initial sample (—), 5 min O₃ (—), 10 min O₃ (—), 15 min O₃ (—), 20 min O₃ (—) and 30 min O₃ (—). Ultrasound pre-treatment: 300 kHz and 40 W. O₃ injection: 7.4 mg O₃/L_{gas}. 50 mg HA/L initial sample.

concentration obtained with ultrasound. This could explain the difference in TOC removal of HA solutions between ultrasound pre-treatment (<5%) and O₃ treatment (>40%) shown in Fig. 5, where the effect of a 30 min sonication pre-treatment is shown to have negligible effect on the subsequent ozonation process. During the first 25 min of ozonation, a sharp decrease in TOC is accompanied by a fast reduction in pH from 7 to 4. At pH between 7 and 4, both HO• and molecular O₃ would take part in the oxidation process of compounds due to a fast decomposition of O₃ [34,35]. When the pH reached 4 or below, a considerable reduction in TOC removal rate was observed, where oxidation and mineralisation of HAs would come primarily from the action of molecular O₃ (significantly lower oxidation potential compared to HO• [36]).

All this indicates that O₃ would have played a minor role in the reduction of TOC compared to HO•. That would explain why after approximately 25 min of ozonation TOC removal rate decreased significantly with only a 12% additional removal between 30 min and 2 h with 7.4 mg O₃/L_{gas} injected compared to a 30% reduction in the first 30 min (Fig. 5). The formation of refractory compounds during ozonation could have also reduced further mineralisation of HAs [10]. Therefore, ozonation time was set at 30 min for further experiments.

3.4.2. Effect on fluorescence spectra

The evolution of fluorescence is compared in Fig. 7a (ultrasound pre-treatment without and with post O₃ treatment) and 7b (O₃ treatment alone). In both cases, a significant decrease in 460–550 nm emission band was observed (Fig. S5) indicating decomposition of HAs which are composed of aromatic groups, alcohols and phenols, as well as ketone and aldehyde groups [37]. However, there was as negligible impact from US pre-treatment and ozone dosage. There is a second region consisting of proteins with a maximum emission peak at 405 nm. In this region, the fluorescence intensity for ozonation alone resulted in a consistently lower intensity compared to ozonation with ultrasonic pre-treatment, indicating higher degradation of proteins by ozone (Fig. S6).

In the fluorescence emission region between 340 and 410 nm, there is a notable increase in the intensity when ultrasound pre-treatment is applied compared to only ozonation. This could indicate that ozonation after ultrasonic pre-treatment led to an increase in the more biodegradable part of the humic structure (340–410 nm) [38], increasing: (i) the protein-like group that consists of xenobiotic compounds, (ii) the tryptophan-like group constituted by low molecular weight compounds and (iii) the tyrosine-like group composed of free molecules or molecules bound to higher molecular weight proteins, as well as peptides [39]. On the other hand, with O₃ treatment alone, although the humic region decreased its intensity in the same way as with the ultrasonic pre-

treatment, an increase in the 340–390 nm band (Fig. S7) that could indicate a greater oxidation of the humic structure into other by-products with more biodegradable characteristics was not observed.

3.4.3. HPSEC analysis

Fig. 7c and d correspond to HPSEC chromatography of the ultrasonic pre-treatment followed by the ozonation process. In general terms, a gradual reduction in humic substances up to 80% was observed along with the generation of lower molecular weight oxidation compounds such as carboxylic acids and esters. HAs were broken down into lower molecular weight compounds (from >640 kDa to ~20 kDa) during the treatment process as shown by Fig. 7d. No major difference was found in the formation of lower molecular weight compounds during ozonation with and without ultrasonic pre-treatment (Fig. S8).

3.4.4. FTIR analysis

With regards to the FTIR analysis, Fig. 8 shows the infrared spectra obtained during the ozonation process with ultrasonic pre-treatment. A decrease in the transmittance intensity at 1620–1630 cm⁻¹ indicates a decrease in aromatic groups after 30 min O₃ injection [40,41], likely due to O₃ being highly selective towards complex aromatic compounds [30]. The decrease in aromaticity is consistent with the decrease in SUVA₂₅₄ and fluorophore concentration in the emission band of 405–461 nm observed in fluorescence analyses [42,43]. After the hydroxylation and opening of aromatic rings, a slow oxidation of oxygenated saturated compounds likely took place [44] as observed in the decrease of the corresponding to aromatic ethers [45]. Along with the 1260 cm⁻¹ band functional group, a decrease of the 1030 cm⁻¹ band (alcohols) was also observed, that together with the phenolic groups slowly decreased with increasing reaction time [41]. It is also highlighted the decrease in transmittance in the 1720 cm⁻¹ band (corresponding to the C=O vibration tension) attributable to ketone groups and carboxylic acids. Above this wavelength there is a progressive decrease in compounds capable of absorbing in FTIR, which leads to a convergence of the spectra whatever the treatment. Finally, after the decrease in aromatic groups, alcohols, phenols, ketones and aldehydes, a greater abundance of the C-O tension bands (1220 cm⁻¹) was observed, corresponding to carboxylic acids [40,44]. These carboxylic groups increased during the reaction, being results in line with those observed in fluorescence analyses. In contrast, the 805 cm⁻¹ band would be attributable to tri- and tetrasubstituted aromatic rings which remained unchanged throughout the treatment [46]. This fraction could correspond to the residual humic structure observed in the fluorescence and which by its nature has a recalcitrant character that is difficult to eliminate (more aliphatic and polar) [41]. Finally, the intensity at 1540 and 1384 cm⁻¹ would correspond to the formation of nitrogen groups caused by O₃ injection [47].

4. Conclusions

Ultrasonic pre-treatment significantly lowered fluorescence emission intensity from HA samples with all the frequencies under study. 31% fluorescence reduction in λ max (470 nm) was obtained applying 300 kHz and 40 W (50 mg HA/L) for 30 min, measuring also changes in molecular structure. The subsequent 30 min ozonation reduced TOC down to 13.5 mg/L (36%) after applying 7.4 mg O₃/L_{gas}. O₃ injection also led to the production of oxidation by-products of lower molecular weight, reducing colour (85%) and SUVA₂₅₄ (70%) significantly. However, ultrasonic pre-treatment did not substantially improve the performance of ozonation treatment. Thus, similar results for TOC, colour and SUVA₂₅₄ were obtained with ozonation-alone experiments. In contrast, significant variations in the fluorescence emission of the samples were detected, despite reaching the same degree of mineralisation either with or without ultrasonic pre-treatment. In such a way, the use of ultrasound would be more advantageous if adequately combined with simple or biological adsorption processes.

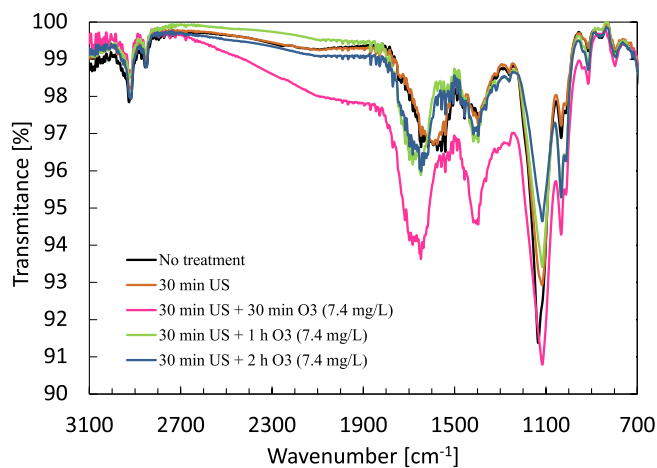


Fig. 8. FTIR spectra in the region between 3100 and 700 cm⁻¹ of initial sample (50 mg HA/L and 400 mL), 30 min ultrasound (US, 300 kHz and 40 W), 30 min US + 30 min O₃ (7.4 mg O₃/L_{gas}), 30 min US + 1 h O₃ and 30 min US + 2 h O₃.

CRediT authorship contribution statement

Pello Alfonso-Muniozgueren: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization. **Cristian Ferreira:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization. **Elodie Richard:** Investigation, Data curation. **Madeleine Bussemaker:** Resources, Writing - review & editing, Supervision, Funding acquisition. **José Ignacio Lombrana:** Conceptualization, Methodology, Resources, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition. **Judy Lee:** Conceptualization, Methodology, Resources, Writing - review & editing, Visualization, Project administration, Funding acquisition.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ultsonch.2020.105359>.

References

- G.R. Aiken, D.M. McKnight, R.L. Wershaw, P. MacCarthy, An introduction to humic substances in soil, sediments and water, in: A.W.I. Publication (Ed.)1985, pp. 1–9.
- V. Naddeo, V. Belgiorno, R.M.A. Napoli, Behaviour of natural organic matter during ultrasonic irradiation, *Desalination* 210 (2007) 175–182.
- Y.-S. Ma, J.-G. Lin, Effect of pre-sonication on removal of organic matters resulting from chlorinated humic acids, *Water Sci. Technol.* 38 (1998) 253–260.
- Y. Nagata, K. Hirai, H. Bandow, Y. Maeda, Decomposition of hydroxybenzoic and humic acids in water by ultrasonic irradiation, *Environ. Sci. Technol.* 30 (1996) 1133–1138.
- D. Chen, Z. He, L. Weavers, C. Yu-ping, W. Harold, P. Hatcher, Sonochemical reactions of dissolved organic matter, *Res. Chem. Intermed* 30 (2004) 735–753.
- Y. Liu, Q. Wang, S. Zhang, J. Lu, S. Yue, NOM reactivity with chlorine in low SUVA water, *J. Water Supply: Res. Technol.-Aqua* 60 (2011) 231–239.
- R.J. Wood, J. Lee, M.J. Bussemaker, A parametric review of sonochemistry: control and augmentation of sonochemical activity in aqueous solutions, *Ultrason Sonochem.* 38 (2017) 351–370.
- S. Manickam, N. Abidin, S. Parthasarathy, I. Alzorqi, E.H. Ng, T.J. Tiong, R. L. Gomes, A. Ali, Role of H₂O₂ in the fluctuating patterns of COD (chemical oxygen demand) during the treatment of palm oil mill effluent (POME) using pilot scale triple frequency ultrasound cavitation reactor, *Ultrason Sonochem* 21 (2014) 1519–1526.
- E. Villaroel, J. Silva-Agrede, C. Petrier, G. Tabora, R.A. Torres-Palma, Ultrasonic degradation of acetaminophen in water: Effect of sonochemical parameters and water matrix, *Ultrason. Sonochem.* 21 (2014) 1763–1769.
- K. Kusakabe, S. Aso, J.-I. Hayashi, K. Isomura, S. Morooka, Decomposition of humic acid and reduction of trihalomethane formation potential in water by ozone with U.V. irradiation, *Water Research*, 24 (1990) 781–1.
- H. Miao, W. Tao, Ozonation of humic acid in water, *J. Chem. Technol. Biotechnol.* 83 (2008) 336–344.
- G.L. Amy, C.J. Kuo, R.A. Sierka, Ozonation of humic substances: Effects on molecular weight distributions of organic carbon and trihalomethane formation potential, *Ozone Sci. Eng.* 10 (1988) 39–54.
- J. Larose, A. Grasmick, P. Blondeau, S. Elmaleh, J.P. Legeron, Elimination of humic materials, *Ozone Sci. Eng.* 4 (1982) 79–89.
- T.M. Olson, P.F. Barbier, Oxidation kinetics of natural organic matter by sonolysis and ozone, *Water Res.* 28 (1994) 1383–1391.
- L.K. Weavers, M.R. Hoffmann, Sonolytic decomposition of ozone in aqueous solution: mass transfer effects, *Environ. Sci. Technol.* 32 (1998) 3941–3947.
- L.K. Weavers, F.H. Ling, M.R. Hoffmann, Aromatic compound degradation in water using a combination of sonolysis and ozonolysis, *Environ. Sci. Technol.* 32 (1998) 2727–2733.
- L. Stepniak, E. Stanczyk-Mazanek, M. Kusiak, Ultrasounds and ozone in removal of humic substances from water, *Inżynieria i Ochrona Środowiska* 15 (2012) 143–154.
- C. Ferreira, N. Villota, J.I. Lombrana, M.J. Riveiro, An efficient catalytic process for the treatment of genotoxic aniline wastewater using a new granular activated carbon-supported titanium dioxide composite, *J. Cleaner Prod.* 228 (2019) 1282–1295.
- J. Reijenga, A. van Hoof, A. van Loon, B. Teunissen, Development of Methods for the Determination of pKa Values, *Anal Chem Insights* 8 (2013) 53–71.
- A.A. Helal, G.A. Murad, A.A. Helal, Characterization of different humic materials by various analytical techniques, *Arabian J. Chem.* 4 (2011) 51–54.
- F. Mijangos, F. Varona, N. Villota, Changes in solution color during phenol oxidation by fenton reagent, *Environ. Sci. Technol.* 40 (2006) 5538–5543.
- E.J. Hart, A. Henglein, Free radical and free atom reactions in the sonolysis of aqueous Iodide and formate solutions, *J. Phys. Chem.* 89 (1985) 4342–4347.
- A. Weissler, H.W. Cooper, S. Snyder, Chemical effect of ultrasonic waves: oxidation of potassium iodide solution by carbon tetrachloride, *J. Am. Chem. Soc.* 72 (1950) 1769–1775.
- E. Naffrechoux, S. Chanoux, P.J. Suptil, Sonochemical and photochemical oxidation of organic matter, *Ultrason. Sonochem.* 7 (2000) 255–259.
- P. Kanthale, M. Ashokkumar, F. Grieser, Sonoluminescence, sonochemistry (H₂O₂ yield) and bubble dynamics: frequency and power effects, *Ultrason Sonochem.* 15 (2008) 143–150.
- J. Lee, Importance of Sonication and Solution Conditions on the Acoustic Cavitation Activity, in: M. Ashokkumar (Ed.), *Handbook of Ultrasonics and Sonochemistry*, Springer Science + Business Media, Singapore, 2016, pp. 137–175.
- S. Parthasarathy, R.R. Mohammed, C.M. Fong, R.L. Gomes, S. Manickam, A novel hybrid approach of activated carbon and ultrasound cavitation for the intensification of palm oil mill effluent (POME) polishing, *J. Cleaner Prod.* 112 (2016) 1218–1226.
- P.R. Gogate, Application of cavitation reactors for water disinfection: current status and path forward, *J Environ Manage* 85 (2007) 801–815.
- R.A. Torres-Palma, J. Gibson, I.G. Droppo, P. Seto, R. Farnood, Surfactant-assisted sono-breakage of wastewater particles for improved UV disinfection, *Water Air Soil Pollut.* 228 (2017) 106.
- C. von Sonntag, U. von Gunten, *Chemistry of Ozone in Water and Wastewater Treatment: From Basic Principles to Applications*, IWA Publishing, 2012.
- T. Nöthe, H. Fahlenkamp, C.V. Sonntag, Ozonation of Wastewater: Rate of Ozone Consumption and Hydroxyl Radical Yield, *Environmental Science & Technology*, 43 (2009) 5990–5995.
- R.M. Hozalski, E.J. Bouwer, S. Goel, Removal of natural organic matter (NOM) from drinking water supplies by ozone-biofiltration, *Water Sci. Technol.* 40 (1999) 157–163.
- E.S. Melin, H. Ødegaard, The effect of biofilter loading rate on the removal of organic ozonation by-products, *Water Res.* 34 (2000) 4464–4476.
- M.-O. Buffle, J. Schumacher, S. Meylan, M. Jekel, U. von Gunten, Ozonation and advanced oxidation of wastewater: effect of O₃ dose, pH, DOM and HO• scavengers on ozone decomposition and HO• generation, *Ozone Sci. Eng.* 28 (2006) 247–259.
- P. Alfonso-Muniozgueren, M. Hazzwan Bohari, A. Sicilia, C. Avignone-Rossa, M. Bussemaker, D. Saroj, J. Lee, Tertiary treatment of real abattoir wastewater using combined acoustic cavitation and ozonation, *Ultrason. Sonochem.* 64 (2020), 104986.
- S.C. Ameta, P.B. Punjabi, A. Kumar, R. Ameta, *Advanced Oxidation Processes: Basics and Applications*, in: D.G. Rao, R. Senthilkumar, J.A. Byrne, S. Feroz (Eds.) *Wastewater Treatment: Advanced Processes and Technologies*, IWA publishing 2012, pp. 46.
- N.P. Sanchez, A.T. Skeriotis, C.M. Miller, Assessment of dissolved organic matter fluorescence PARAFAC components before and after coagulation–filtration in a full scale water treatment plant, *Water Res.* 47 (2013) 1679–1690.
- N. Hudson, A. Baker, D. Ward, D.M. Reynolds, C. Brunson, C. Carliell-Marquet, S. Browning, Can fluorescence spectrometry be used as a surrogate for the Biochemical Oxygen Demand (BOD) test in water quality assessment? An example from South West England, *Sci. Total Environ.* 391 (2008) 149–158.
- S.A. Baghoth, S.K. Sharma, G.L. Amy, Tracking natural organic matter (NOM) in a drinking water treatment plant using fluorescence excitation–emission matrices and PARAFAC, *Water Res.* 45 (2011) 797–809.
- R. Gracia, J.L. Aragües, J.L. Ovelleiro, Study of the catalytic ozonation of humic substances in water and their ozonation byproducts, *Ozone Sci. Eng.* 18 (1996) 195–208.
- F.J. Rodríguez, P. Schlenger, M. García-Valverde, Monitoring changes in the structure and properties of humic substances following ozonation using UV–Vis, FTIR and 1H NMR techniques, *Sci. Total Environ.* 541 (2016) 623–637.
- M. Sillanpää, A. Matilainen, T. Lahtinen, Chapter 2 – Characterization of NOM, in: M. Sillanpää (Ed.) *Natural Organic Matter in Water*, Butterworth-Heinemann2015, pp. 17–53.
- X. Zhong, C. Cui, S. Yu, Identification of oxidation intermediates in humic acid oxidation, *Ozone Sci. Eng.* 40 (2018) 93–104.
- R. Gracia, S. Cortés, J. Sarasa, P. Ormad, J.L. Ovelleiro, Heterogeneous catalytic ozonation with supported titanium dioxide in model and natural waters, *Ozone Sci. Eng.* 22 (2000) 461–471.

- [45] H.-C. Kim, M.-J. Yu, Characterization of aquatic humic substances to DBPs formation in advanced treatment processes for conventionally treated water, *J. Hazard. Mater.* 143 (2007) 486–493.
- [46] M. Derrien, S.R. Brogi, R. Goncalves-Araujo, Characterization of aquatic organic matter: assessment, perspectives and research priorities, *Water Res* 163 (2019), 114908.
- [47] S. Preis, I. Panorel, S. Llauger Coll, I. Kornev, Formation of nitrates in aqueous solutions treated with pulsed corona discharge: the impact of organic pollutants, *Ozone Sci. Eng.* 36 (2014) 94–99.