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Removal of contaminants of emerging concern from the supernatant of anaerobically digested sludge by O_3 and O_3/H_2O_2 : Ozone requirements, effects of the matrix, and toxicity

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ABSTRACT

Digestate is a rich source of nutrients that can be applied in agricultural fields as fertilizer or irrigation water. However, most of the research about application of digestate have focused on its agronomic properties and neglected the potential harm of the presence of contaminants of emerging concern (CECs). Advanced oxidation processes (AOPs) have proved to be effective for removing these compounds from drinking water, yet there are some constraints to treat wastewater and digestate mainly due to their complex matrix. In this study, the feasibility to remove different CECs from digestate using O_3 and O_3/H_2O_2 was assessed, and the general effect of the matrix in the oxidation was explained. While the lab-scale ozonation provided an ozone dose of 1.49 mg O_3 /mg DOC in 5 h treatment, almost all the compounds were removed at a lower ozone dose of maximum 0.48 mg O_3 /mg DOC; only ibuprofen required a higher dose of 1.1 mg O_3 /mg DOC to be oxidized. The digestate matrix slowed down the kinetic ozonation rate to approximately 1% compared to the removal rate in demineralized water. The combined treatment (O_3/H_2O_2) showed the additional contribution of H_2O_2 by decreasing the ozone demand by 59–75% for all the compounds. The acute toxicity of the digestate, measured by the inhibition of *Vibrio fischeri* luminescence, decreased by 18.1% during 5 h ozonation, and by 34% during 5 h O_3/H_2O_2 treatment. Despite the high ozone consumption, the ozone dose (mg O_3 /mg DOC) required to remove all CECs from digestate supernatant was in the range or lower than what has been reported for other (waste-)water matrix, implying that ozonation can be considered as a post-AD treatment to produce cleaner stream for agricultural purposes.

1. Introduction

Anaerobic digestion (AD) plants, acting as circular bioeconomic hubs, can recover valuable renewable energy and nutrients from multiple organic waste streams. The AD biological process converts solid organic waste to biogas. The remaining sludge (digestate) is further dewatered and the digestate supernatant, due to its high content of nitrogen and phosphorus, can be used as irrigation water (Wang and Lee, 2021). However, the potential presence of contaminants of emerging concern (CECs), could hinder the utilization of digestate supernatant in the agricultural field (Minh et al., 2009; Edith et al., 2019; Gurmessa et al., 2020). Despite the well-established agronomic benefits of

digestate (Koszel and Lorencowicz, 2015; Simon et al., 2015), various research indicated that AD processes do not significantly contribute to CECs removal (Widyasari-Mehta et al., 2016; Gros et al., 2019). The presence of pharmaceutical compounds such as antibiotics, non-steroidal anti-inflammatory drugs (NSAIDs), and also hormones, most of them exhibiting endocrine disrupting properties, have been reported in treated wastewater, sludge, and digestate in municipal/agro-industrial wastewater treatment plants (WWTPs) (Peng et al., 2006; Petrie et al., 2015). In the liquid digestate from several biogas plants, for example, antibiotics were detected in a wide range of concentrations, for instance 38.5 µg/L for oxytetracycline (Yang et al., 2022), 120 µg/L for tetracycline (Kasumba et al., 2020) and 66,400 µg/L for chlortetracycline (Nurk et al., 2019). The endocrine disrupting

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Abbreviations

17- β -EST	17- β -Estradiol	DOX	Doxycycline
AD	Anaerobic digestion	DW	Demineralized Water
ARBs	Antibiotic-Resistant Bacteria	IBU	Ibuprofen
AOD	Applied Ozone Dose	NSAIDs	Nonsteroidal Anti-Inflammatory Drugs
AOPs	Advanced Oxidation Processes	NPX	Naproxen
BPA	Bisphenol A	OTC	Oxytetracycline
CEC	Contaminants of Emerging Concern	SMN	Sulfamethazine
CTC	Chlortetracycline	SMX	Sulfamethoxazole
DIC	Diclofenac	TCN	Tetracycline
DOC	Dissolved Organic Carbon	TOC	Total Organic Carbon
DOM	Dissolved Organic Material	TOD	Transferred Ozone dose
DON	Dissolved Organic Nitrogen	UOD	Utilized Ozone dose
		WWTPs	Wastewater Treatment Plants

effects on living organisms and the development of antibiotic-resistant bacteria (ARBs), among others, are examples of the ecological and hazardous impacts that they may have (Ouda et al., 2021). However, liquid digestate is currently applied to agricultural fields without any post-AD treatment for the removal of CECs. As such, an increasing attention must be put on their removal before the liquid digestate is applied to soils.

Ozone-based AOPs have been successfully applied to remove CECs from drinking water and as an advance treatment in WWTPs (Qu et al., 2015; Bui et al., 2016; Hansen et al., 2016; de Oliveira et al., 2020; Asghar et al., 2022). Ozone can oxidize most of the organic contaminants either by directly attacking electron-rich sites of the target compounds or by indirectly producing hydroxyl radicals ($\text{OH}\cdot$) (Miklos et al., 2018) that will later oxidize the target compound. Wang et al. (2023) showed an increase in indirect mechanism and an enhance in micropollutant removal from biotreated landfill leachate by adding H_2O_2 to the ozonation treatment (Wang et al., 2023). In another study, Lee et al. (2023) indicated that the combination of $\text{O}_3/\text{H}_2\text{O}_2$ in treating wastewater effluent improves the removal of ozone-resistance micropollutant, and prevents the formation of some toxic by-product including bromate (Lee et al., 2023).

Yet, the application of ozone-based AOPs to treat water samples with high load of organic matter such as digestate supernatant is rather limited. The possible challenges in treating digestate supernatant with ozonation in compare with the above-mentioned (waste-)water matrix may include both the high load of dissolved organic matter (DOM), as well as the presence of carbonate species competing for the $\text{OH}\cdot$ radicals (Buffle et al., 2006; Asghar et al., 2022). In a study to track the matrix effects on the oxidation of pharmaceuticals during ozonation, it was observed that the removal of pharmaceutical varied in different water matrices. For instance, the residual concentration of phenacetin, at an initial concentration of 1 μM and after dosing 1 mg/L of ozone was 0.71 μM for a wastewater sample with TOC of 13.2 mg/L, and 0.9 μM for a wastewater with TOC of 22.9 mg/L. The same pattern was observed for different ozone dose ranging between 0.5 and 5 mg/L. Thus, it was concluded that the higher the load of organic matter, the lower the amount of oxidant available for the target micropollutant; i.e., necessitating higher oxidant concentration for achieving the target removal efficiency (Javier Benitez et al., 2009). Cruz-Alcalde et al. (2020) showed a competitive effect exhibited by the organic matter on the micropollutant removal during ozonation of wastewater at different concentrations of organic matter. According to their study, the amount of ozone needed to remove refractory micropollutants from wastewater increased from 19 mg/L in the sample with DOC of 6.6 mg/L to 48 mg/L in the sample with DOC of 21.3 mg/L (Cruz-Alcalde et al., 2020).

1.1. Research gap

Digestate supernatant is currently applied in agricultural field without further treatment for CECs removal. Adsorption, filtration, and AOPs are just a few of the technologies used to remove CECs. Nevertheless, AOPs are favoured since adsorption and filtering do not degrade CECs but rather transfer them from one phase to another. Regarding the AOPs, although ozonation is a potential technology for removing CECs, a review of the literature revealed a lack of data on its application to treat digestate supernatant. The most recent review papers shows the effect of the organic matter on increasing the ozone demand in different wastewater matrix, yet the range of DOC in the studied wastewaters varied between 5 and 48 mg/L (Asghar et al., 2022). Treating the current digestate supernatant in this research with a DOC around 1200 mg/L can significantly increase the ozone dose required. The problem accelerates by considering the high load of carbonate species in digestate supernatant (1950 mg CaCO_3/L), that not only slows down the ozone decomposition and affects the direct mechanism of CECs removal (Katsoyiannis et al., 2011), but it also scavenges the $\text{OH}\cdot$ radicals, limiting the exposure of CECs to ozone and $\text{OH}\cdot$ radicals. Furthermore, the high load of ammonia in the digestate supernatant (700 mg/L) can stoichiometrically consume a substantial portion of ozone and produces nitrite and nitrate that have the potential to significantly scavenge the $\text{OH}\cdot$ radicals (Lado Ribeiro et al., 2019). Having mentioned these challenges and considering the high cost and energy required for ozone generation, it is important to determine the ozone dose required for CECs removal from digestate supernatant before considering ozonation as a potential post-AD treatment to remove these compounds.

As such and with the main goal of contributing to improve the potential handling and reuse of the treated digestate supernatant, it is essential to assess how the physicochemical characteristics of the digestate (the water matrix) can affect or interfere with the removal of CECs. Therefore, this study aimed to assess (i) the removal of certain CECs present in digestate supernatant, (ii) the specific ozone dose required to achieve a target removal of CECs, (iii) the potential effects of the digestate matrix on the removal of CECs, (iv) the comparison of the CECs removal performance when using O_3 versus $\text{O}_3/\text{H}_2\text{O}_2$, and (v) the toxicity removal of the digestate supernatant during O_3 and $\text{O}_3/\text{H}_2\text{O}_2$ oxidation.

Based on the type of the digestate, the most consumed veterinary antibiotics for food processing animals was chosen to assess in this study. Furthermore, NSAIDs and bisphenol A (a known endocrine disruptive compound) have been added to the selected list of compounds. This selection has been made considering their frequent occurrence in different types of sludge and their threat to the environment.

2. Materials and methods

2.1. Chemicals

Doxycycline hyclate (DOX), tetracycline (TCN), chlortetracycline hydrochloride (CTC), oxytetracycline (OTC), sulfamethoxazole (SMX), sulfamethazine (SMN), 17 β -Estradiol (17- β -EST), ibuprofen (IBU), naproxen (NPX), bisphenol A (BPA), and diclofenac sodium (DIC) were purchased from Sigma Aldrich, Chemie GmbH, Germany. A stock solution of each compound was prepared in methanol (HPLC Grade) at a concentration of 4 g/L. Thereafter, to conduct each ozonation test, a working solution was prepared in demineralized water (DW) reaching a final concentration of 10 mg/L of each compound. Before each experiment, the working sample (prepared either in a DW matrix or in the digestate supernatant matrix) was spiked with the working solution reaching a concentration of 100 μ g/L of each target compound.

2.2. Digestate collection and sample preparation

Grab samples of the digestate (20 L) were collected from the outflow valve of a digester in the biogas plant Mpisiritsas, Kozani in Greece. The biogas plant with an annual energy generation of 854 MWh is situated near the town of Servia in Kozani. The feedstock of the plant is composed of animal faeces, urine and manure (including spoiled straw) and corn silage. Generally, the feedstock consists of 50 m³ (tonnes) of pig waste and 2 tonnes of corn silage. The digester is operated at mesophilic condition, and the digestate is currently used in land application by local farmers who provide the corn silage feedstock.

Prior to each experiment, the collected digestate was pasteurized at 70 °C for 3 h and separated using a centrifuge at 4800 rpm for 20 min followed by a series of sieves and vacuum filtration with 1.2 μ m (GFC, Whatman), and 0.45 μ m pore size filters (Whatman). Later, the maximum transferred ozone in the laboratory-scale ozone set-up was determined during set-up calibration (Text S1), and since the ozone transferred was limited, the digestate supernatant was diluted to reach a dissolved organic carbon (DOC) concentration of approximately 275 mg/L. With the applied dilution, it was possible to supply the ozone dose around 1 mg O₃/mg DOC, and track the removal process in the short-term experiment (5 h). The working sample (Table 1) was spiked with CECs up to a final concentration of 100 μ g/L of each compound. Prior to the experiments with digestate, no more chemicals including buffering compounds were added to the sample, and pH were tracked throughout the experiments. As for demineralized water, the initial pH was increased to 8.5 by adding NaOH (1 M).

2.3. Set-up and ozone experiment

Ozone experiments were conducted using a bubble column reactor in a semi-continuous mode. The set-up consisted of an ozone generator with activated alumina air DSC dryer (Trailigaz LABO, France), a PVC reactor made of transparent acrylate with a 2.6 L (51.75 cm height and 8 cm inner diameter) capacity equipped with a humidifier (DH3b, BMT

MESTECHNIK, Berlin, Germany), and a diffuser with 5 cm diameter, which was installed at the bottom of the reactor occupying 40% of the bottom area of the reactor (Fig. 1). The off-gas was continuously captured by the humidifier and destructed in a catalytic ozone destruction equipment. Prior to the experiment, the gaseous ozone concentration in the inlet was adjusted to 20.5 $\pm$ 0.2 mg/L and kept constant throughout the experiments. The inlet and off-gas ozone concentrations were monitored and recorded by an online sensor (Ozone analyser BMT 964, BMT MESTECHNIK GmbH, Stanhnsdorf, Germany). The flowrate of the air was set at 350 L/h, and the given pressure, current and voltage were 0.78 bar, 0.7 A, and 210 V (Power = 0.132 kW), respectively, to maintain a steady ozonated air flowrate of 51 $\pm$ 2 L/h throughout the experiments. The resulting ozone stream was introduced in the reactor via the coarse bubble diffuser located at the bottom of the reactor. The dissolved O₃ concentration in the liquid phase was monitored by an online sensor (Krypton KO₃ ozone in water analyzer, Dr. A. Kuntze GmbH Meerbusch, Germany); in addition, the ozone concentration was also monitored taking regular samples and conducting the Indigo method (Bader and Hoigné, 1981). A minimum of 2.5 L of the sample (either with the DW matrix or with the digestate supernatant) was introduced into the reactor to minimize the headspace in the reactor, and the experiment was carried out in ambient temperature (20 °C). To assess the kinetic of the oxidation process, samples were withdrawn at different contact times (0, 0.25, 0.5, 0.75, 1, and after 5 h); after the samples were taken, the residual ozone/OH $\cdot$ was quenched using Na₂S₂O₃ (at a final concentration of 80 mg/L), and kept refrigerated at -18 °C before the analyses.

2.4. Ozone mass balance and data treatment

To evaluate the ozone mass balance and ozone mass transfer, three parameters including the Applied Ozone Dose (AOD), the Transferred Ozone Dose (TOD), and the Utilized Ozone Dose (UOD), were determined using Eq. (1) to Eq. (3)

$$AOD \left(\frac{\text{mg O}_3}{\text{h}} \right) = Q_g \left(\frac{\text{L}}{\text{h}} \right) \cdot C_{O_3-g-in} \left(\frac{\text{mg}}{\text{L}} \right) \quad (1)$$

$$TOD \left(\frac{\text{mg O}_3}{\text{h}} \right) = Q_g \left(\frac{\text{L}}{\text{h}} \right) \cdot \left(C_{O_3-g-in} \left(\frac{\text{mg}}{\text{L}} \right) - C_{O_3-g-out} \left(\frac{\text{mg}}{\text{L}} \right) \right) \quad (2)$$

$$UOD \left(\frac{\text{mg O}_3}{\text{h}} \right) = TOD - \left(\left(C_{O_3-L} \left(\frac{\text{mg}}{\text{L}} \right) \cdot V_L(L) \right) / t(h) \right) \quad (3)$$

where, AOD represents the applied ozone mass into the reactor in a fraction of time, Q_g is the flow rate of the ozonated gas, C_{O_3-g-in} is the ozone concentration in the gas phase entering the reactor recorded by the online sensor. TOD is the cumulative transferred ozone dose into the liquid in a fraction of time, $C_{O_3-g-out}$ is the concentration of the ozone in the off-gas recorded by the online sensor before the off-gas goes to the ozone destructor, UOD is the utilized (consumed) ozone mass in the reactions, C_{O_3-L} is the dissolved ozone concentration that is recorded by online liquid sensor, and V_L is the volume of the liquid in the reactor.

To compare the CECs removal efficiency from the digestate with the CECs removal efficiency from other (waste-)water matrices, the O₃ dose required for at least 90% removal of the target compounds was determined as per Eq. (4) (Antoniou et al., 2013). By dividing the O₃ dose per the initial DOC of each sample, the obtained specific ozone dose (mg O₃/mg DOC) can be compared across different (waste-)water samples.

$$\log \frac{C}{C_0} = - \frac{DO_3}{DDO_3} \leftrightarrow C = C_0 \times 10^{-\frac{DO_3}{DDO_3}} \quad (4)$$

$$\text{Where, } DO_3 = \frac{UOD}{V_L} \quad (5)$$

Eq. (4) describes the ratio of each compound to its initial concentration (C and C₀) after certain O₃ consumption (DO₃) during the

Table 1
Physicochemical characterization of the working sample.

Parameter	Dimension	Value	std
COD	mg/L	987	11.8
DOC	mg/L	275	8.6
TN	mg TN-N/L	212	6
TKN	mg TKN-N/L	208	9.7
Ammonia	mg NH ₄ -N/L	172	7.1
Nitrite	mg NO ₂ -N/L	n.d ^a	–
Nitrate	mg NO ₃ -N/L	n.d	–
Alkalinity	mg CaCO ₃ /L	489	11.2
pH	–	8.5	–

^a not detected.

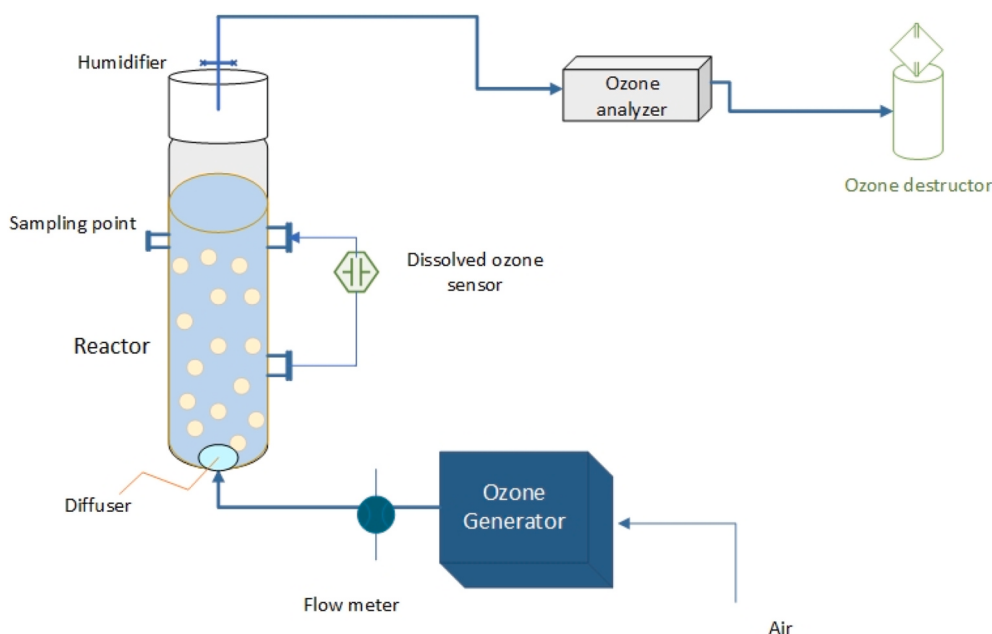


Fig. 1. Schematic of ozone set-up.

ozonation time. By data fitting to Eq. (4), DDO_3 was determined for each compound. DDO_3 is the decadic ozone dose required for 90% removal of each compound, and it is a compound specific parameter in each water matrix which is independent of CEC concentration and depends on the ozone decomposition in each water matrix (Hansen et al., 2016).

For the specific DDO_3 , the DDO_3 obtained from Eq. (4) was divided by the initial DOC. This dimensionless term specifies the O_3 -degradability of the compounds as follows: if $\frac{DDO_3}{DOC} < 0.7$ then the compound is easily degradable, if $0.7 < \frac{DDO_3}{DOC} < 1.4$ then the compound is moderately degradable, and if $\frac{DDO_3}{DOC} > 1.4$ then the compounds is O_3 -recalcitrant (Hansen et al., 2010, 2016; Antoniou et al., 2013).

Furthermore, since AOPs are electric-energy-intensive (Miklos et al., 2018), for economic purposes, it is crucial to assess the operational energy consumption (Yang et al., 2021). The treatment efficiency was evaluated using electrical energy per order (E_{EO}) recommended by IUPAC and described by Bolton et al. (2001). E_{EO} (kWh/m^3) is defined as the electrical energy consumption to remove the contaminant by one order of magnitude (90%) in $1 m^3$ of water (Eq. (6))

$$E_{EO} = \frac{1000 \times W \times t}{V \times \log\left(\frac{C_0}{C_t}\right)} \quad (6)$$

where, W is the power of the system (kW), t is the treatment time (h), V is the volume of the water (L), C_0 and C_t are the concentration (mg/L) of the target contaminant at time 0 and time t , respectively.

2.5. Analytical methods

The following equipment and methods were applied for the analytical determination of the parameters of interest: ammonia (NH_4-N) based on Standard Methods (NEN 6742) (APHA, 1992) using a spectrophotometer (PerkinElmer, UV-Vis Lambda 365, the Netherlands), Total Kjeldahl Nitrogen (TKN) by a TKN apparatus equipped with a Kjeldtherm digester (Gerhardt, Germany) and a distiller (Gerhardt, Vapodest, Germany), and the dissolved organic nitrogen (DON-N) by deducting the concentration of the background ammonia from TKN. Total dissolved nitrogen (TN) and dissolved organic carbon (DOC) were measured by TOC analyser (Shimadzu, the Netherlands), alkalinity (mg $CaCO_3/L$) was measured by automatic titration (Metrohm, 848 Titriano plus, Applicon, the Netherlands), and NO_3-N was measured by ion

chromatography (ICS-1000, Dionex, the Netherlands).

The CECs analysis was subcontracted to Laboratorios Tecnológicos de Levante, Valencia, Spain (Certificate n° 121/LE1782) where the following methods were applied: For BPA, the internal standard Bisphenol A D16 was added to the 10 times diluted sample, and the derivatization was done in basic medium ($pH > 9$ with NaOH) with acetic anhydride. The analytes were extracted using the SBSE (twister) technique (12 h, 1500 rpm). The twister was collected and analysed by thermal desorption in GC-QQQ (GC chromatograph, Agilent 7890) equipped with Triple Quadrupole Mass Spectrometer (QQQ Agilent 7000C), with MPS Autosampler Gerstel, using a Sapiens Column 5-MS (30 m 0.25 mm 0.25 μm), with the flow of 1.2 mL/min. The sample linear range was between 0.1 and 10 $\mu g/L$ with detection limit of 0.1 $\mu g/L$.

For antibiotics, the sample was stirred and filtered by filter of 13 mm PTFE Hydrophilic, 0.45 μm Teknokroma (Ref TR-F1-0021). The filtrate was collected in vial and analysed by direct injection into high-performance liquid chromatography (Agilent HPLC Agilent 1260), equipped with triple quadrupole-mass spectrometer (Agilent QQQ Agilent 6460). The applied column was Eclipse plus C18 (2.1 $\times$ 100 mm, 1.8 μm) with mobile Phase A: Water 0.1% formic acid, and mobile Phase B: acetonitrile 0.1% formic acid with the flow of 0.3 mL/min. The linear range for CIP, ERY, SMX and SMN was between 0.1 and 100 ppb, and for TCN, CTC, DOX, and OTC between 0.5 and 500 ppb with detection limit of 0.5 $\mu g/L$.

For NSAIDs and hormone, the sample was stirred and filtered by filter of 13 mm PTFE Hydrophilic, 0.45 μm Teknokroma (Ref TR-F1-0021), and the filtrate was collected in vial and analysed by direct injection into the same HPLC-QQQ but equipped with Poroshell 120 Phenyl-Hexyl column (3.0 $\times$ 100 mm, 2.7 mm) with mobile phase A: Water 0.1 mM ammonium fluoride, mobile phase B: methanol 0.1 mM ammonium fluoride with the flow of 0.4 mL/min. The linear range for 17- β -EST, DIC, NPX and IBU was between 0.1 and 100 ppb with detection limit of 0.1 $\mu g/L$. All the target CECs were detected with analysis recovery between 86 and 110%.

2.6. Kinetic studies

Previous studies have applied the chemical kinetic method based on Eq. (7) to predict the removal of a variety of CECs during O_3 treatment

(Gomes et al., 2017)

$$-\frac{dC}{dt} = k_{O_3}[O_3][C] + k_{OH\cdot}[OH\cdot][C] \quad (7)$$

where, C is the concentration (M) of the target compounds and k_{O_3} and $k_{OH\cdot}$ are the apparent rate constant of the reaction of each compound with ozone, and $OH\cdot$, respectively. Since $OH\cdot$ is produced from the ozone decomposition in the liquid, its concentration is proportional to the concentration of O_3 , thus, the simplified and integrated form of Eq. (7) can be shown as Eq. (8) for comparison purposes:

$$-\ln \frac{C_t}{C_0} = k_{obs} \int_0^t O_3 dt \quad (8)$$

Where, k_{obs} is $k_{O_3} + R_{CT} k_{OH\cdot}$ and R_{CT} is $\int_0^t C_{OH\cdot} dt / \int_0^t O_3 dt$ (He et al., 2022).

$\int_0^t O_3 dt$ was determined by integrating DO_3 in Eq. (5) to ozonation time, and k_{obs} was calculated by fitting the data to Eq. (8).

2.7. MicroTox® test

MicroTox® test with *Vibrio fisheries* is a highly sensitive, reproducible, and internationally accepted method which has been standardized for measuring toxicity (Libralato et al., 2010). The test is based on the luminescence light emission of the marine organism *Vibrio fisheries*. The osmotic adjustment solution, diluent, and solo reagent shot vials containing the organism were purchased from MicroLAN b.v. (the Netherlands). The organism was exposed to the untreated and treated samples and the acute toxicity assay was carried out by measuring the inhibition of the light emission after 5, 15 and 30 min exposure time via Toxicity-meter (MicroTox M500, SDI, MicroLAN, the Netherlands).

2.8. Research approach

In this study, digestate supernatant was treated with O_3 and O_3/H_2O_2 in a continuous ozonation bubble column reactor. To determine the ozone dose required for removal of the target CEC, kinetic rate, effect of the matrix, effect of the chain initiator (H_2O_2), and toxicity, five experiments were conducted (Table 2).

To achieve the objectives of this study, the ozonation of the CECs in DW was conducted to obtain the removal rate of the target compound without the effect of interferences such as organic matter (Exp. 1). Then, under the same operational condition, the ozonation was conducted to treat digestate supernatant spiked by the same CECs (Exp. 2). By comparing the kinetic rate and ozone consumption in Exp. 1 and 2, it was possible to determinate the competition effect of the digestate matrix on the removal. Thereafter, the optimum dosage of H_2O_2 was determined in Exp. 3, and the experiments were repeated under the same operational condition by adding hydrogen peroxide to the reactor to treat demineralized water spiked with CECs (Exp. 4) and digestate supernatant spiked with CECs (Exp. 5), and the potential effect of

hydrogen peroxide on the removal of the CECs was assessed. During digestate supernatant treatment in Exp. 2 and 5, the changes in the matrix component such as alkalinity, DOC and organic/inorganic nitrogen was determined to study the potential competition of the matrix component on ozone consumption. At the end, the toxicity of the treated and untreated digestate in Exp. 2 and Exp. 5 was determined using the Microtox® test.

3. Results and discussion

3.1. Ozone mass balance and mass transfer in demineralized water and digestate

The O_3 profile in the gas and liquid phase as a function of treatment time and the ozone mass balance during ozone treatment for demineralized water (Exp. 1) and digestate supernatant (Exp. 2) is depicted in Fig. 2. The same profile for O_3/H_2O_2 treatment applying for demineralized water (Exp. 4) and digestate supernatant (Exp. 5) is shown in Fig. 3. The dissolved O_3 was observed only in the DW treated by O_3 , but for the digestate it remained zero throughout the entire treatment.

While treating DW (Exp. 1), since no other chemicals besides CECs was in the sample, the dissolved O_3 was detectable before 15 min (Fig. 2a). In the rest of the experiments (Exp. 2, 4 and 5) however, the dissolved O_3 remained zero or was negligible during the 5 h ozonation, indicating that all the transferred ozone was consumed in ozone reaction with target compounds or organic material in general. Considering the negligible concentration of dissolved ozone, the ozone transfer yield (TOD/AOD) is the main parameter to characterize the behaviour of each water matrix (either DW or digestate) towards ozone and to show the reactivity of the matrix components. The transfer yield in Figs. 2 and 3 shows that; (i) a two-phase of ozonation can be identified in all the O_3 profiles in Figs. 2 and 3, with the first phase up to 15 min that the transfer yield increased, and the second phase after 15 min that the transfer yield gradually dropped until the end of the ozonation time; (ii) comparing Fig. 2b and d shows a higher transfer yield in digestate (53%) in 15 min, in compare to the maximum transfer yield in DW (3.5%) that implies more reactions of ozone in the digestate matrix due to the high load of organic and inorganic material competing for ozone consumption. The same trend can be observed by comparing Fig. 3b–d; (iii) the transfer yield in O_3/H_2O_2 treatment (Fig. 3) is higher than in O_3 treatment (Fig. 2) both in DW (transfer yield: 14%) and digestate supernatant (transfer yield of 70%), which can be explained by more reaction of ozone with H_2O_2 .

The first phase of ozonation (the first 15 min) represents the fast reactions between O_3 and organic matter that increases the O_3 demand and results in higher transfer yield. Although dissolved O_3 was still not detected in the second phase, a drop in the transfer yield indicates a slower oxidation of more resistant/refractory organic matters. Due to the lack of information about the ozone transfer in complex matrix such as digestate supernatant, comparison the transfer yield in this study with other digestate was not possible. Yet, the same pattern of transfer was

Table 2
Outline of the experiments.

Experiment	Sample	Treatment	O_3 Mass balance	CEC analysis	Toxicity (<i>Vibrio fisheries</i>)	Objective
Exp. 1	Demineralized water spiked with CECs	O_3	+	+	–	Comparing CECs removal from DW and digestate to assess the effect of the matrix
Exp. 2	Digestate supernatant spiked with CECs	O_3	+	+	+	
Exp. 3	Digestate supernatant	O_3 + different ratio of $H_2O_2:O_3$	+	–	–	Optimization the ratio of H_2O_2/O_3
Exp. 4	Demineralized water spiked with CECs	O_3/H_2O_2	+	+	–	Comparing Exp.4 and Exp.5 for the effect of the matrix, Comparing Exp. 2 and Exp. 5 for the effect of H_2O_2
Exp. 5	Digestate supernatant spiked with CECs	O_3/H_2O_2	+	+	+	

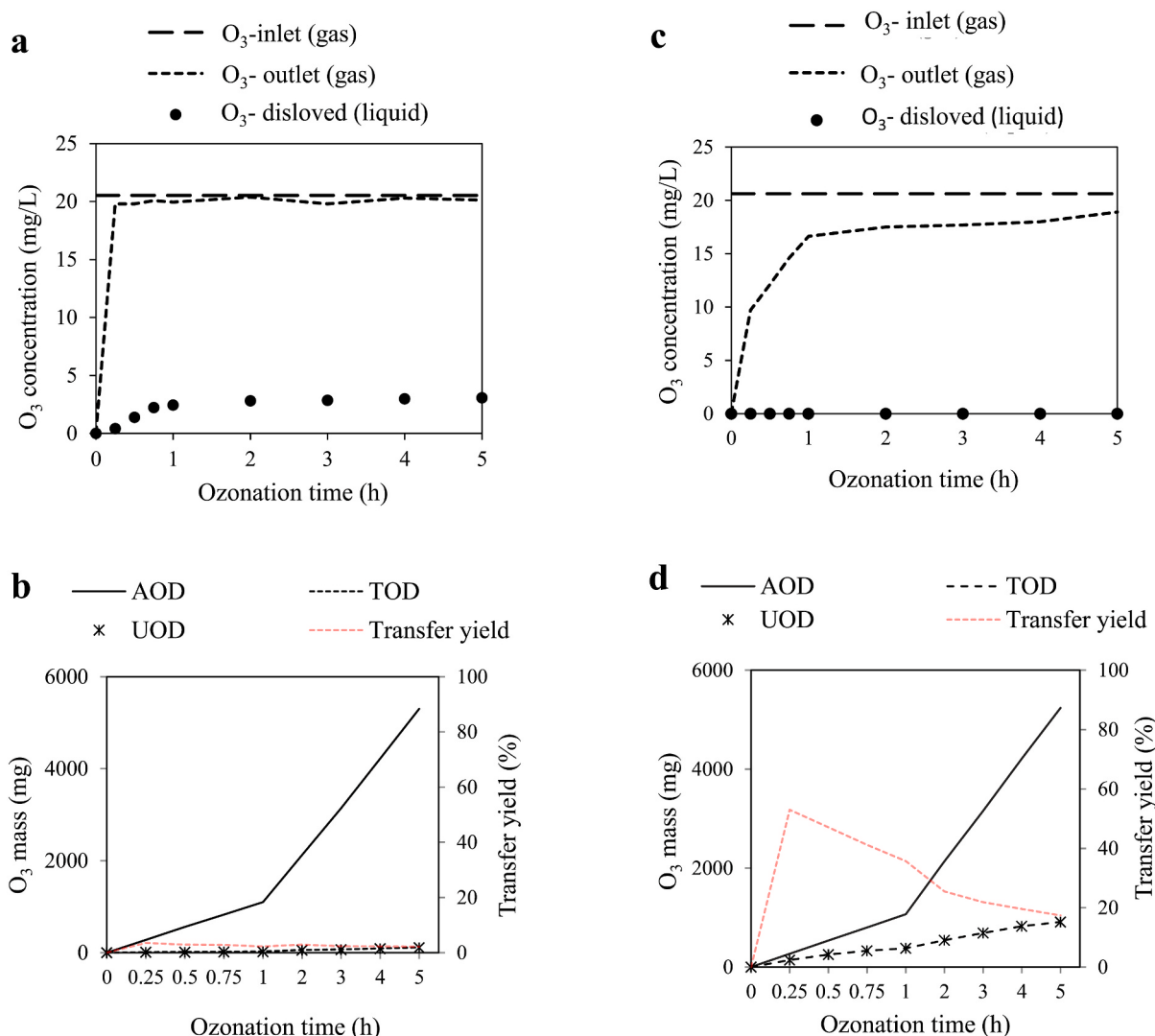


Fig. 2. Gaseous and liquid O₃ profile, mass balance and mass transfer during 5 h O₃ treatment; (a) O₃ profile in DW spiked with contaminants of emerging concern (Exp. 1), (b) mass balance in DW spiked with contaminants of emerging concern, (c) O₃ profile in digestate spiked with contaminants of emerging concern (Exp. 2), and (d) mass balance in digestate spiked with contaminants of emerging concern. (Initial ozone concentration: 20.5 ± 0.2 mg/L, ozone flow rate: 51 ± 2 L/h, Sample volume: 2.6 L).

observed during the ozonation of other water matrix. For instance, Yang et al. (2021) reported up to 90% transferred yield in 20 min ozonation of landfill leachate containing 142 mg/L DOC, which is higher than the transfer yield observed in digestate supernatant. Yet, they also reported the same decreasing pattern of the transfer yield after 20 min despite the increase of the ozone dose (Yang et al., 2021). The less transfer yield in our study in compare with their study might be due to the matrix effect of the digestate that hampers the ozone reactions, and also the presence of more refractory compounds in the digestate supernatant.

3.2. Removal of contaminants of emerging concern by O₃ and O₃/H₂O₂

Dividing the ozone consumption (DO₃) by the DOC content of digestate supernatant, the specific ozone dose was calculated, and the CEC removal as a function of the specific ozone dose was determined and shown in Fig. 4 for both O₃ and O₃/H₂O₂ treatment. The optimization of H₂O₂/O₃ ratio (Exp. 3), for the current water matrix with DOC of 275 mg/L, can be seen in Text S2 and Fig. S1. Applying O₃ and O₃/H₂O₂ treatment on DW with a DOC of 11.2 mg/L (Exp. 1 and Exp. 4), all the CECs except IBU were removed from DW in the first 15 min at a specific O₃ dose of 0.26 mg O₃/mg DOC by O₃ treatment, and in the first

5 min at a specific O₃ dose of 0.16 mg O₃/mg DOC by O₃/H₂O₂ (ratio of H₂O₂/O₃: 2.5) treatment (data not shown). Applying the same operational conditions like for DW, the complete removal of all the evaluated CECs from the digestate supernatant matrix occurred in a 45 min period at a specific ozone dose of 0.51 mg O₃/mg DOC (Exp. 2). IBU showed the lowest removal efficiency, requiring a 5 h treatment with specific ozone dose of 1.11 mg O₃/mg DOC for 90% removal. Nevertheless, by adding hydrogen peroxide (Exp. 5), the removal efficiencies below the detection limits were observed in less than 15 min at specific ozone dose of 0.27 mg O₃/mg DOC. Also, for IBU, the removal efficiency increased, and 96% removal was achieved in 0.5 h at specific O₃ dose of 0.51 mg O₃/mg DOC.

To compare the CEC removal efficiency from digestate with other (waste-)water matrix in previous studies, the DDO₃ and specific DDO₃ for 90% removal was determined by fitting the data for each CEC to Eq. (4). Furthermore, the E_{EO} to remove each compound was calculated by using Eq. (6) and added to Table 3. As it can be observed in Table 3, the interference of the digestate component on the removal efficiency in ozone treatment significantly (p -value = $0.0002 < 0.05$) increased the specific ozone dose for all the compounds (e.g.; from 0.26 to 0.32 mg O₃/mg DOC for DOX, and from 0.52 to 1.11 mg O₃/mg DOC for IBU)

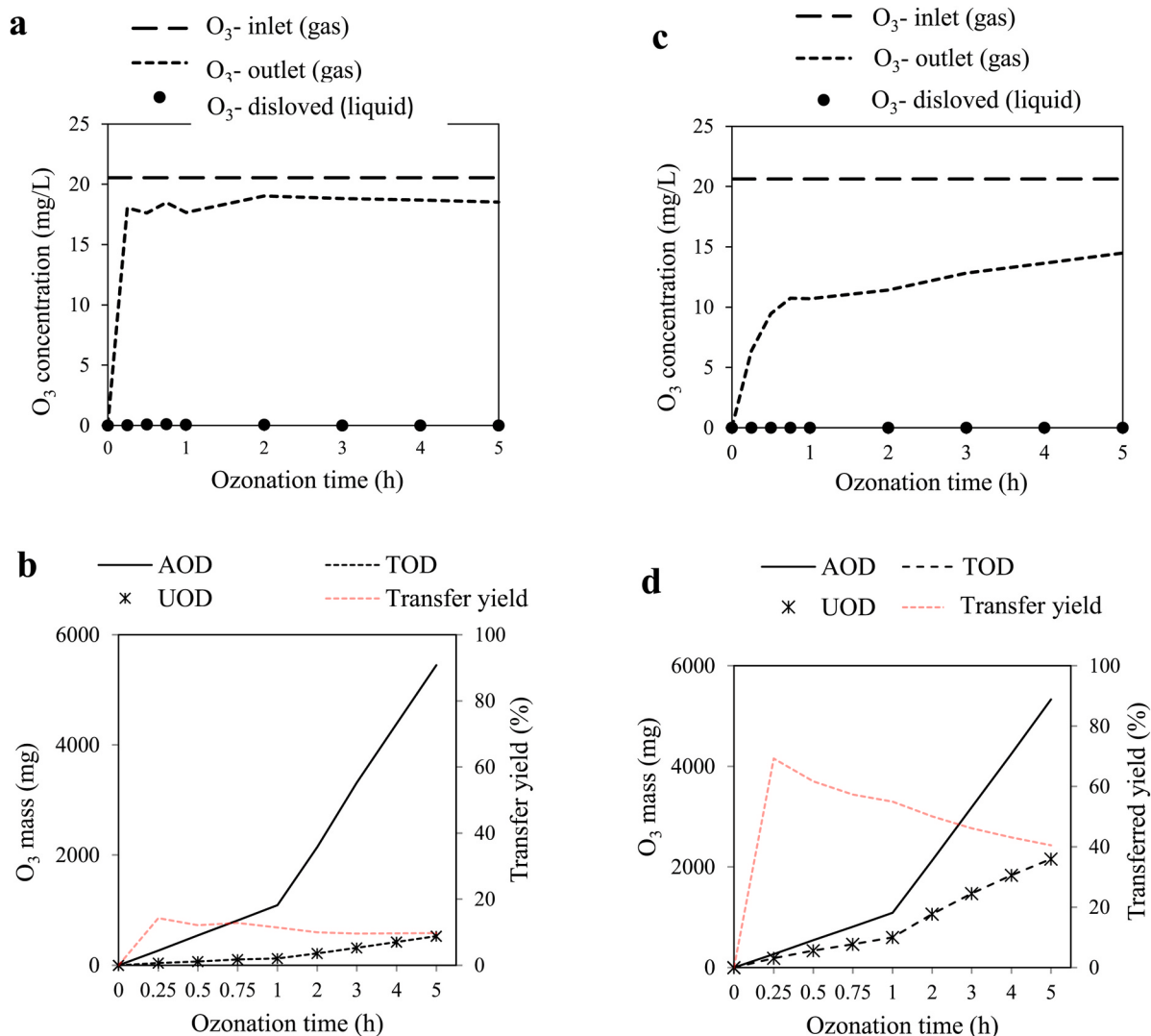


Fig. 3. Gaseous and liquid O₃ profile, mass balance and mass transfer during 5 h O₃/H₂O₂ treatment; (a) O₃ profile in DW spiked with contaminants of emerging concern (Exp. 4), (b) mass balance in DW spiked with contaminants of emerging concern, (c) O₃ profile in digestate spiked with contaminants of emerging concern (Exp. 5), and (d) mass balance in digestate spiked with contaminants of emerging concern. (Initial ozone concentration: 20.5 ± 0.2 mg/L, ozone flow rate: 51 ± 2 L/h, Sample volume: 2.6 L).

compared to DW. Besides, the energy consumption to achieve the same removal in digestate increased (e.g.; from 7.9 to 18.5 kWh/m³ for DOX, and from 57.3 to 132 kWh/m³ for IBU). Adding H₂O₂ decreased the O₃ demand for the removal of the target compounds for instance, for the most recalcitrant CEC in this study, IBU, the specific ozone dose for 90% removal, decreased to 0.46 mg O₃/mg DOC, which was 41.5% less than the ozone dose required in ozone treatment alone. The same trend was observed for all CECs where the specific ozone dose was significantly, with a p-value of 10⁻⁴ < 0.05, lower than the one in ozone treatment.

The specific DDO₃ can be applied to compare the oxidation of each compound in other water matrix (like drinking water or wastewater) (Buffle et al., 2006; Hansen et al., 2016), and to categorize the compounds as easily degradable, moderately degradable and persistent (Antoniou et al., 2013). Based on the data obtained from Table 3, all the evaluated compounds are O₃-degradable except for IBU. Although no data is available for removing CECs from digestate supernatant, comparing the data in Table 3 to previous studies shows that in spite of the complex matrix of the digestate supernatant, the specific DDO₃ is comparable with previously reported data in the literature for wastewater, surface, and groundwater. Antoniou et al. (2013) reported a specific O₃ dose of 0.55–0.77 g O₃/g DOC for easily degradable

compounds including diclofenac and sulfamethoxazole in secondary effluent wastewater (Antoniou et al., 2013). For the removal of the same compounds in an WWTP upgraded with post-ozonation, Hollender et al. (2009) reported a specific ozone dose of 0.47 g O₃/g DOC (Hollender et al., 2009). In the current study, the specific DDO₃ for diclofenac and sulfamethoxazole was 0.35 and 0.38 mg O₃/mg DOC, respectively. For more refractory compounds, such as ibuprofen a specific DDO₃ higher than 1 g O₃/g DOC for secondary effluent treatment was reported (Bahr et al., 2007). For ibuprofen removal from hospital wastewater, a specific ozone dose of 1.3 g O₃/g DOC (Hansen et al., 2016), and from secondary effluent 1.61 g O₃/g DOC (Antoniou et al., 2013) were also reported. In our study, ibuprofen was removed using a specific DDO₃ of 1.11 mg O₃/mg/DOC. The specific ozone dose for the removal of the target CEC in our study compared with other studies was either in the range or slightly lower although the digestate supernatant contains higher concentrations of organic matter rather than the previous studies (e.g. of between 5.2 and 18 mg DOC/L reported by Hansen et al. (2016), Antoniou et al. (2013), and Bahr et al. (2007)). Thus, the stoichiometry of DDO₃/DOC for CEC removal is not affected by digestate matrix.

The E_{EO} value for 90% removal of CECs from DW was between 4.8 and 57.3 kWh/m³ which was significantly lower (p-value = 0.001 <

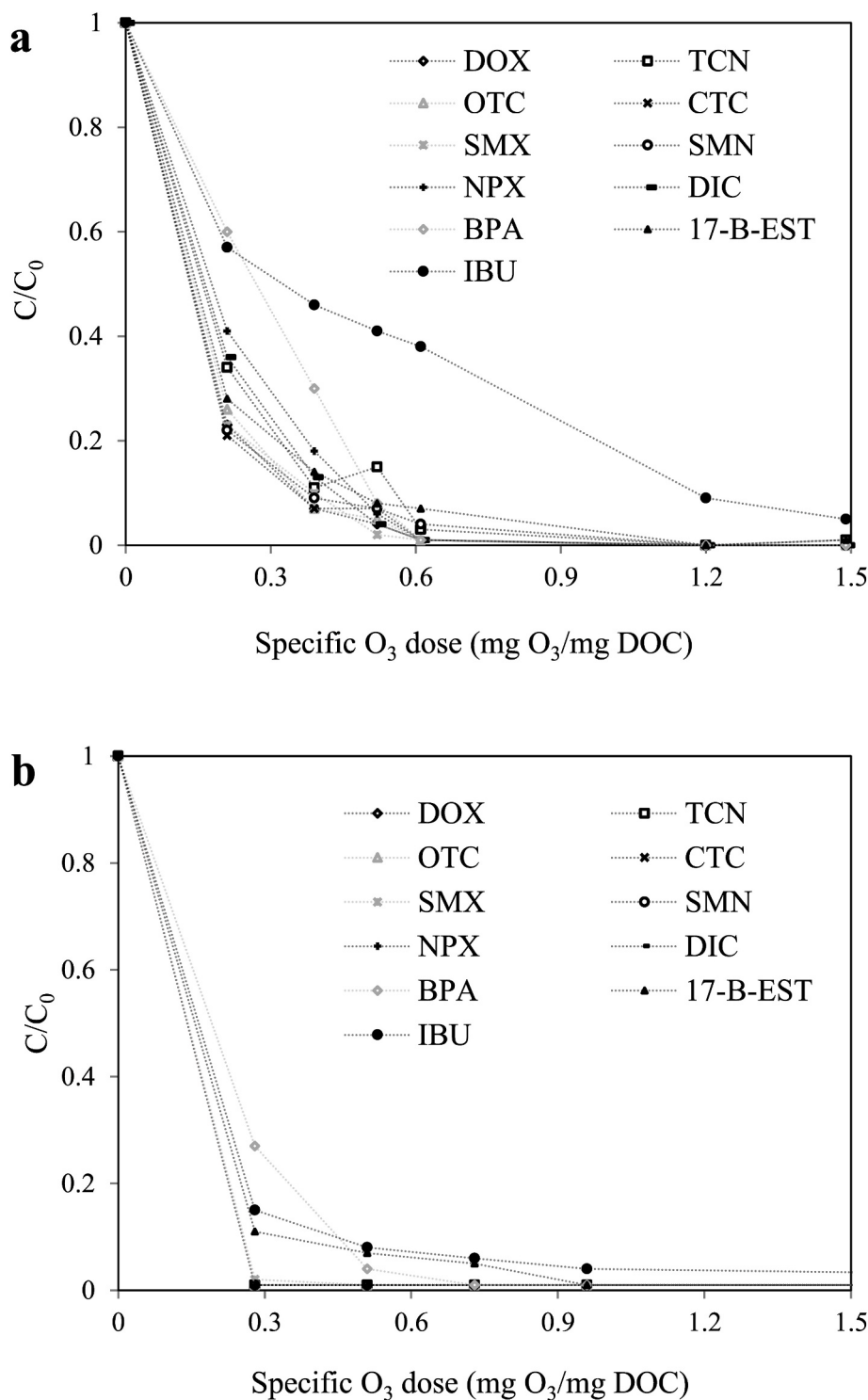


Fig. 4. Removal of various contaminants of emerging concern from digestate supernatant in different specific ozone dose in (a) O₃ and (b), O₃/H₂O₂ treatment (H₂O₂/O₃: 2.5) (C₀ and C refers to the initial concentration of each compound and its concentration when a specific O₃ dose was applied within the 5 h experiment).

0.05) than the E_{EO} in digestate (25.2–132 kWh/m³). The higher E_{EO} in digestate shows the effect of the matrix component that most likely compete with the target compounds for ozone consumption. Adding H₂O₂ decreased the E_{EO} required to achieve the same removal (e.g. from 27.5 to 13.2 kWh/m³ for TCN, and from 132 to 24.1 kWh/m³ for IBU) due to the contribution of OH· radical that increases the CEC removal by more oxidation. Since E_{EO} value depends on various variables including the efficiency and power of ozone generator, water matrix, and pH among others, the reported E_{EO} in the literature varies widely by several

order of magnitude (between 0.001 and 10 kWh/m³ (Miklos et al., 2018)). For instance, an E_{EO} between 0.14 and 1.1 kWh/m³ for removing 17-B-EST from biologically treated sewage was reported by Hansen et al. (2010). Pisarenko et al. (2012) reported an E_{EO} range between 0.022 kWh/m³ for DIC to 0.393 kWh/m³ for IBU in O₃ treatment of MBR-filtrate wastewater. In another study a range of 1.4–5.4 kWh/m³ for removing the trace organic contaminants from water was reported (Yang et al., 2021). The E_{EO} in the current study both in DW and digestate was higher in compare with reported values in the

Table 3O₃ dose required for 90% removal of the target compounds from digestate supernatant.

Ozone	DW				Digestate				
	DO ₃ (mg/L)	DDO ₃ (mg/L)	DDO ₃ /DOC (mg O ₃ /mg DOC)	E _{EO} (kWh/m ³)		DO ₃ (mg/L)	DDO ₃ (mg/L)	DDO ₃ /DOC (mg O ₃ /mg DOC)	E _{EO} (kWh/m ³)
DOX	3.56	3	0.26	7.9	106	89.6	0.32		18.5
TCN	3.56	3.7	0.32	7.9	106	110.7	0.4		27.5
OTC	3.56	3.2	0.28	5.3	106	93.8	0.34		18.5
CTC	3.56	3.1	0.27	14.3	106	92.1	0.35		18.5
SMX	3.56	3.5	0.31	5.3	106	105.4	0.38		26.4
SMN	3.56	3.5	0.31	4.8	106	103.1	0.37		25.2
NPX	3.56	2.8	0.25	4.8	142	112.9	0.41		31.7
DIC	3.56	2.5	0.22	4.8	142	98.1	0.35		29
BPA	3.56	3.3	0.29	4.8	142	132.6	0.48		36.1
17-β-EST	3.56	3.3	0.29	4.8	142	130.6	0.47		34.3
IBU	7.84	5.9	0.52	57.3	410	305.9	1.11		132

Ozone/ H ₂ O ₂	DW				Digestate				
	DO ₃ (mg/L)	DDO ₃ (mg/L)	DDO ₃ /DOC (mg O ₃ /mg DOC)	E _{EO} (kWh/m ³)		DO ₃ (mg/L)	DDO ₃ (mg/L)	DDO ₃ /DOC (mg O ₃ /mg DOC)	E _{EO} (kWh/m ³)
DOX	1.86	1.6	0.11	2.1	76	29.2	0.11		13.2
TCN	1.86	1.9	0.13	2.1	76	33.5	0.12		13.2
OTC	1.86	1.6	0.11	2.1	76	29	0.11		13.2
CTC	1.86	1.6	0.11	3.2	76	30.6	0.11		13.2
SMX	1.86	1.9	0.13	2.1	76	39.3	0.14		15.8
SMN	1.86	1.8	0.12	2.1	76	33.5	0.12		13.2
NPX	1.86	1.5	0.10	2.1	76	24.5	0.09		13.2
DIC	1.86	1.3	0.09	2.1	76	23.2	0.08		13.2
BPA	1.86	1.7	0.12	3.7	76	58.2	0.21		18.5
17-β-EST	1.86	1.7	0.12	2.1	76	73.4	0.27		13.2
IBU	5.16	3.9	0.27	14.9	141	125	0.46		24.1

literature which could be explained by the mechanism of ozone generation as well as the matrix effect. The ozone generation mechanism in the current study was less efficient than the mentioned studies since the ozone was produced from air while in those studies the ozone was produced from pure oxygen. Furthermore, the higher organic matter content (e.g. DOC of 275 mg/L in this study in compare with DOC of 6 mg/L for MBR-filtrate wastewater (Pisarenko et al., 2012)) increases the ozone dosage, necessitating a higher E_{EO} to achieve the same removal. However, although the E_{EO} value for digestate was higher than the one reported for other (waste)water in the literature, it is still less energy demanding in compare with other technologies applied for removing CECs for example photocatalysis with E_{EO} of 335 kWh/m³ and ultrasound with E_{EO} of 2616 kWh/m³ (Miklos et al., 2018). As such it has been reported that the AOPs with E_{EO} value between 1 and 100 kWh/m³ might still provide solution for full scale applicability (Miklos et al., 2018).

Nevertheless, the higher ozone consumption (DO₃ in Table 3) in digestate supernatant in compare with other water matrix including DW in this study, implies the inhibition of the digestate supernatant matrix that can affect the kinetic rate of the removal. By fitting the CEC concentration over the ozonation time to Eq. (8), the observed reaction rate constant (k_{obs}) for each compound in DW and digestate supernatant in the presence and the absence of H₂O₂ was determined (Table 4).

In ozone treatment, the observed kinetic rate in digestate supernatant was between 0.09 M⁻¹s⁻¹ (R² = 0.88) for ibuprofen to 0.63 M⁻¹s⁻¹ (R² = 0.91) for doxycycline, which is 1% of the transformation rate obtained from the test in demineralized water. By adding H₂O₂, however, the rate increased; for instance, to 4.2 M⁻¹s⁻¹ (R² = 0.94) for doxycycline and to 1.08 M⁻¹s⁻¹ (R² = 0.96) for ibuprofen. The acceleration and improvement of CEC removal by adding H₂O₂ has been reported in previous studies (Katsoyiannis et al., 2011; Miao et al., 2015; Lado Ribeiro et al., 2019). Lin et al. (2009) reported that adding H₂O₂ at a H₂O₂/O₃ ratio of 5.0 resulted in complete removal of pharmaceuticals from wastewater in 5 min, while, by ozonation alone, the complete removal occurred in 20 min (Lin et al., 2009).

In contrast, in a study for ozonation of pharmaceuticals in hospital

Table 4

Kinetic removal rates of the target compound in demineralized water (DW) and in the digestate supernatant.

Compounds	O ₃		O ₃ /H ₂ O ₂	
	k _{obs} _DW (M ⁻¹ s ⁻¹)	k _{obs} _digestate (M ⁻¹ s ⁻¹)	k _{obs} _DW (M ⁻¹ s ⁻¹)	k _{obs} _digestate (M ⁻¹ s ⁻¹)
Doxycycline	62.9	0.63	361.1	4.2
Tetracycline	59.4	0.52	341.5	3.6
Oxytetracycline	64.4	0.6	370	4.2
Chlortetracycline	31.7	0.6	182.2	4
Sulfamethoxazole	102.4	0.52	588.2	3.1
Sulfamethazine	103.5	0.53	594.2	3.6
Naproxen	106.2	0.34	598.4	5
Diclofenac	108.6	0.39	623.7	5.2
Ibuprofen	9.8	0.09	18.3	0.48
Bisphenol A	81.2	0.29	455.4	1.08
17-β-Estradiol	99.5	0.27	571.5	1.5

wastewater, no observed effect by adding H₂O₂ at a H₂O₂/O₃ ratio of 0.1 was reported (Hansen et al., 2016). The difference can be explained by the applied pH and H₂O₂ dose added to the system. First of all, the applied pH in our study was in the neutral to alkaline range which favours the decomposition of O₃ and generation of OH[•]. In contrast, with the applied pH in their study (5.0–6.25), the decomposition of O₃ is hampered, and the dominant removal mechanism for the target CECs is via the reaction with molecular O₃. The second reason is the higher ratio of H₂O₂ in our study compared to their study. Stoichiometrically, one molecule of H₂O₂ is needed for two molecules of O₃, while in their study the ratio of H₂O₂/O₃ was 0.1, which makes the H₂O₂ the limiting factor in OH[•] generation reactions.

3.3. Potential effect of the molecular structure on CECs removal

The molecular structure of the compounds affects their reaction with ozone, removal efficiency and ozone demand. Ozone selectively reacts with the electron moieties of the compounds. For such reaction, a functional group in the target compound provides the electron dense

moieties for electrophilic reaction of molecular ozone. The electron-rich functional group for the CECs of study includes tertiary amines and phenol group in DOX, TCN, OCT, CTC, secondary amine in SMX, secondary amines and aniline group in SMN, phenol group in BPA and 17- β -EST, and methoxy group in NPX (Table S2). In contrast, IBU, composed of only one ring bound to a carboxyl group. The carboxyl group has the inhibitory effect on electrophilic substitution reaction because it acts as a withdrawal functional group and decreases the density of negative charge on the ring. The carboxylic group can be also

seen in a DIC structure on the phenolic ring, yet the ozone consumption for DIC removal and the reaction rate, categorizes this compound as a fast-degradable CEC. The oxidation of DIC is due to the secondary amine surrounded by the two rings and the electronegativity of the halogens (Cl^-) on one of the rings. Antoniou et al. (2013) has reported the same effect in the removal of poorly degradable compounds (including IBU), suggesting that the contribution of $\text{OH}^\cdot$ radicals can improve the removal rate of this compound (Antoniou et al., 2013). In a pilot study for removing pharmaceuticals from municipal wastewater effluent via

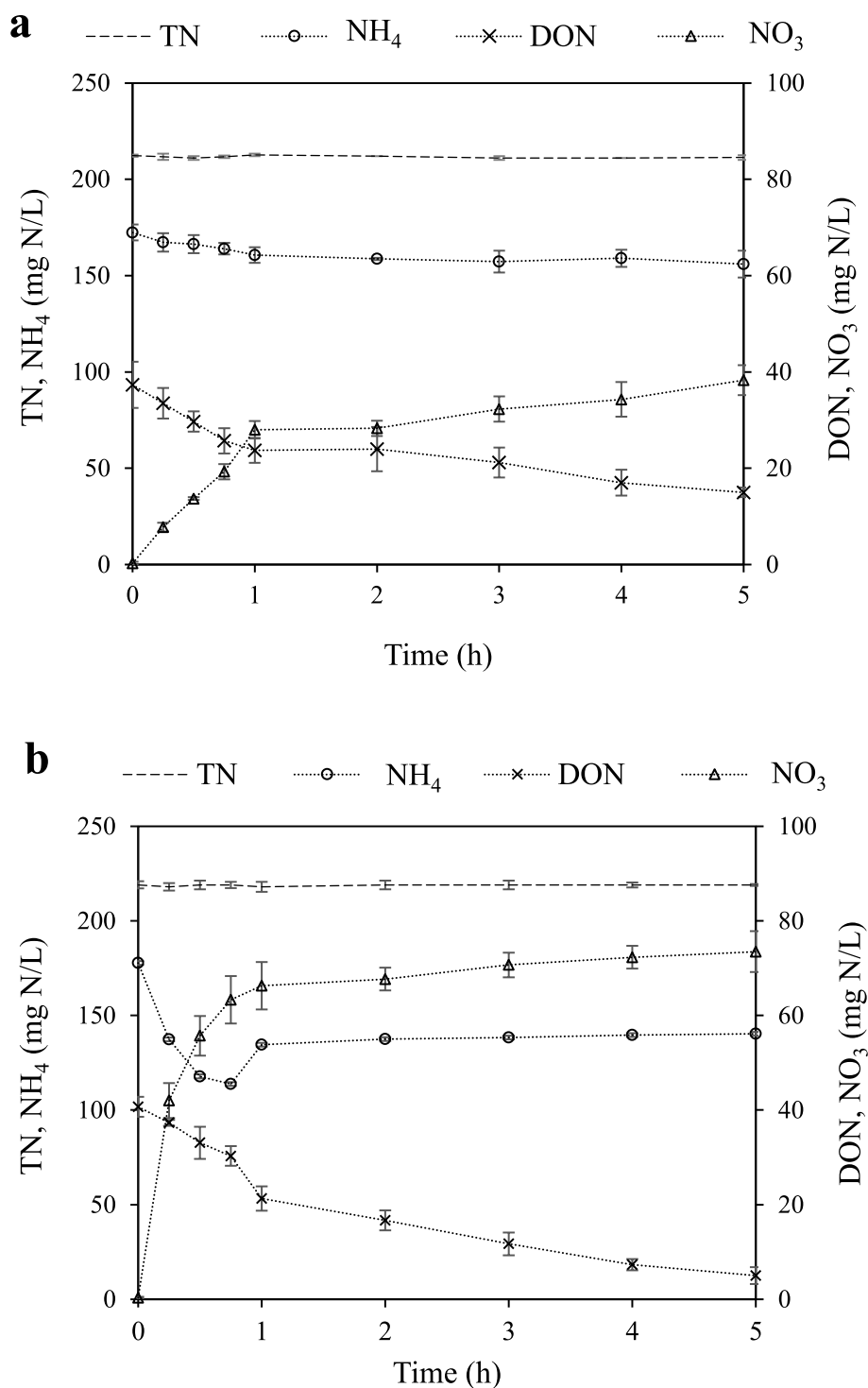


Fig. 5. Oxidation of organic and inorganic nitrogen in digestate supernatant during 5 h (a) O₃ treatment, and (b) O₃/H₂O₂ treatment (Initial ozone concentration: 20.5 ± 0.2 mg/L, ozone flow rate: 51 ± 2 L/h, sample volume: 2.6 L).

ozonation, Huber et al. (2005) also reported that the lack of an electron-rich functional group is the main reason of recalcitrance of IBU towards O_3 treatment, which supports the result of this study regarding the slow reaction of ibuprofen with ozone (Huber et al., 2005).

3.4. Transformation of the matrix components during ozonation of digestate supernatant

The slower removal kinetic rate and higher O_3 demand in the digestate supernatant in compare with DW suggests the potential inhibition/competition effect of the different component in the digestate supernatant. The inhibition effect of the matrix could be due to the O_3 consumption by the matrix components in the direct mechanism, or hampering the formation of $OH\cdot$ radical and scavenging the generated $OH\cdot$ in the indirect mechanism.

The removal of DOC as an indicator for organic matter mineralization turned to be 7% and 29% in O_3 and O_3/H_2O_2 treatment, respectively (data not shown). Despite the low mineralization, Fig. 5 shows that 65% of the dissolved organic nitrogen (DON) was removed during ozonation of the digestate supernatant which suggests a fast reaction of O_3 with the nitrogen fraction present in the organic matter.

The low mineralization (DOC removal) can be explained by the mechanism of the reaction of molecular O_3 with organic compounds. Molecular O_3 reacts selectively with electron rich compounds in an electrophilic reaction by a cycloaddition mechanism, with unsaturated double bond or electron donors' compounds, such as aromatic ring and amines. The decomposition of organic matter may form smaller organic matter and by-product with a lack of double bonds, aromatic groups, and other electron moieties. Thus, in spite of the depletion of the parent compounds, mineralization cannot be expected during ozonation (Gomes et al., 2017). Nevertheless, during 5 h ozonation 38.3 mg NO_3-N/L was formed (Fig. 5a). The formation of NO_3-N during non-catalytic ozonation can be due to the oxidation of inorganic and organic nitrogen. Comparing the NO_3-N yield with the converted NH_4-N (16 mg/L) suggests that stoichiometrically, 42% of the NO_3-N yield could have been originated from the oxidation of ammonia. As such, the rest of the NO_3-N (58%) was produced from the oxidation of N-contained organic matter (DON), that shows the competition of DON with the target compounds for ozone consumption via NO_3-N formation mechanism. In a study for ozonation the wastewater effluent containing 1400 μM NH_4-N and low concentration of NO_3-N (0.5 μM) and DOC of 6.7 mg/L, the contribution of NH_4-N to nitrate production showed a range between 20 and 43% in different O_3 doses, with an average reported value of $32 \pm 7\%$, while DON contributed to production of 68% of the generated NO_3-N (de Vera et al., 2017), which is in line with this study. By adding H_2O_2 to the system, the generated NO_3-N during 5 h ozonation was 73.5 mg/L (Fig. 5b), which is 1.9 times higher than in ozone treatment alone. This can be explained by the contribution of $OH\cdot$ radicals in oxidation of DON and NH_4-N . $OH\cdot$ is a non-selective oxidant that attacks all the organic and inorganic compounds, while ozone selectively attacks only to the electron-rich compounds.

The total alkalinity (mg $CaCO_3/L$) decreased by only 3.6 and 6.4% for O_3 and O_3/H_2O_2 , respectively (Fig. S2), and the pH remained constant at (8.5 ± 0.2) during the 5 h experiment. However, the alkalinity effect on ozonation could be via the indirect mechanism by scavenging the $OH\cdot$ radicals. During the indirect mechanism in ozonation process, formation of $OH\cdot$ radical and the effect of the scavengers could affect the CECs removal efficiency. It has been reported, for instance, that carbonate species and nitrite inhibit radical-based reactions in ozonation and affect the removal of the compounds that have a low reaction rate with molecular ozone (Feng et al., 2016; Asghar et al., 2022). Previous research showed that the main $OH\cdot$ scavengers are DOM (measured as DOC), HCO_3^- , CO_3^{2-} , and nitrite with an apparent reaction rate constant of 8.1×10^4 ($(mg\ C/L)^{-1}s^{-1}$), 8.5×10^6 ($M^{-1}s^{-1}$), 3.9×10^8 ($M^{-1}s^{-1}$), and 1.0×10^{10} ($M^{-1}s^{-1}$), respectively (Lee et al., 2013; Yang et al., 2021). Furthermore, a high load of ammonia in the digestate matrix has

also scavenging effect on $OH\cdot$ radical with a rate constant of 2.3×10^6 ($M^{-1}s^{-1}$) for protonated form at $pH < 7$, and $1.8 \times 10^8\ M^{-1}s^{-1}$ for natural form at $pH > 7$ (Yang et al., 2021). These compounds interfere the conversion rate of O_3 to $OH\cdot$ and consume the $OH\cdot$. The total scavenging rate of each water matrix is usually determined by multiplying these apparent rates to the concentration of the scavengers in each matrix (Yang et al., 2021). In our study, nitrite was not found during the experiments; based on the pH, the majority of alkalinity was in the form of bicarbonate with molar concentration of 0.0489 M, and the carbonate was negligible; and ammonia was in its natural form (NH_3). As such, given the concentration of DOC, alkalinity (in the form of bicarbonate), and ammonia (Table 1), the total $OH\cdot$ scavenging rate turned to be $2.49 \times 10^7\ s^{-1}$, with the contribution of DOC 89% ($2.3 \times 10^7\ s^{-1}$), alkalinity (HCO_3^-) 1.67% ($4.16 \times 10^5\ s^{-1}$), and ammonia 8.88% ($2.2 \times 10^6\ s^{-1}$). Due to the lack of information in the literature, comparing the $OH\cdot$ scavenging rate in different digestate supernatants was not possible. However, the effect of scavengers in other wastewater streams on CECs removal have been widely reported. For instance, a total scavenging rate of $1.9 \times 10^5\ s^{-1}$ was reported during the ozonation of municipal effluent (DOC = 9.6 mg C/L, alkalinity 2.5 mg $CaCO_3/L$, Nitrate = 0.24 mg N/L) (Liu et al., 2020), which is lower than the scavenging rate determined in digestate supernatant, implying the higher inhibition of the matrix in digestate in compare with the wastewater effluent.

3.5. Toxicity (Microtox®)

The inhibition of luminescence emission by organism *Vibrio fisheries* were compared for the samples taken from the reactor in O_3 and O_3/H_2O_2 treatment in Fig. 6.

By increasing the assay time from 5 to 15 and 30 min, the acute toxicity, measured as inhibition of the luminescence emission, increased by an average of 4.9 and 13.5% for O_3 treatment and by an average of 3.75 and 12.6% for O_3/H_2O_2 treatment. In 5 h ozone treatment, the inhibition decreased by 17.3, 14.3, and 18% for assay exposure time of 5, 15, and 30 min. However, the highest inhibition (52–59%) was observed in 0.25 h with the ozone dose of 0.2 mg O_3/mg DOC. A possible explanation is that in lower ozone dose, high toxic transformation by-products might have been formed while with more ozone exposure they were removed. O_3/H_2O_2 treatment was more effective in toxicity removal. As for toxicity assay of 5 min, the inhibition decreased from 39 to 5.7%, which is 1.7 times more than in O_3 treatment. It can be explained by more mineralization in compare to ozone treatment, and as a result less toxic compounds for the organism, i.e. formaldehyde, acetate, and carboxylic acid which are the end by-product of ozonation (Antonioni et al., 2012). In a study for ozonation of biologically treated hospital wastewater with ozone dose between 2.4 and 18 mg/L, an increase in luminescence inhibition of *vibrio fisheries* was reported at ozone dose of 10 mg/L and then the toxicity decreased by dosing more ozone up to 18 mg/L (Tang et al., 2019), which supports the result of the current study. The same increasing pattern was observed for ozonation of biologically treated municipal wastewater when the toxicity measured by the same organism increased at ozone dose of between 0.38 and 0.47 mg O_3/mg DOC (Tang et al., 2020). Yet, A full acute toxicity removal was reported in continuous ozonation of sewage treatment plant effluent by ozone dose of up to 150 mg/L without any observed increase in toxicity (Carbajo et al., 2015). By screening the inhibition of *Vibrio fisheries* in different matrix, Wang et al. (2023), reported that the acute toxicity towards this organism is not easily comparable in different matrix. Since the organism is sensitive to the variety of organic and inorganic pollutants, by-products and pH, the acute toxicity measured by luminescence emission of *Vibrio fisheries* differs in different matrix (Wang et al., 2021). It can explain the difference in toxicity pattern and also the different ozone dose in which an increasing pattern was observed in this study, and in the reported ozonation of municipal and hospital wastewater (Carbajo et al., 2015; Tang et al., 2020).

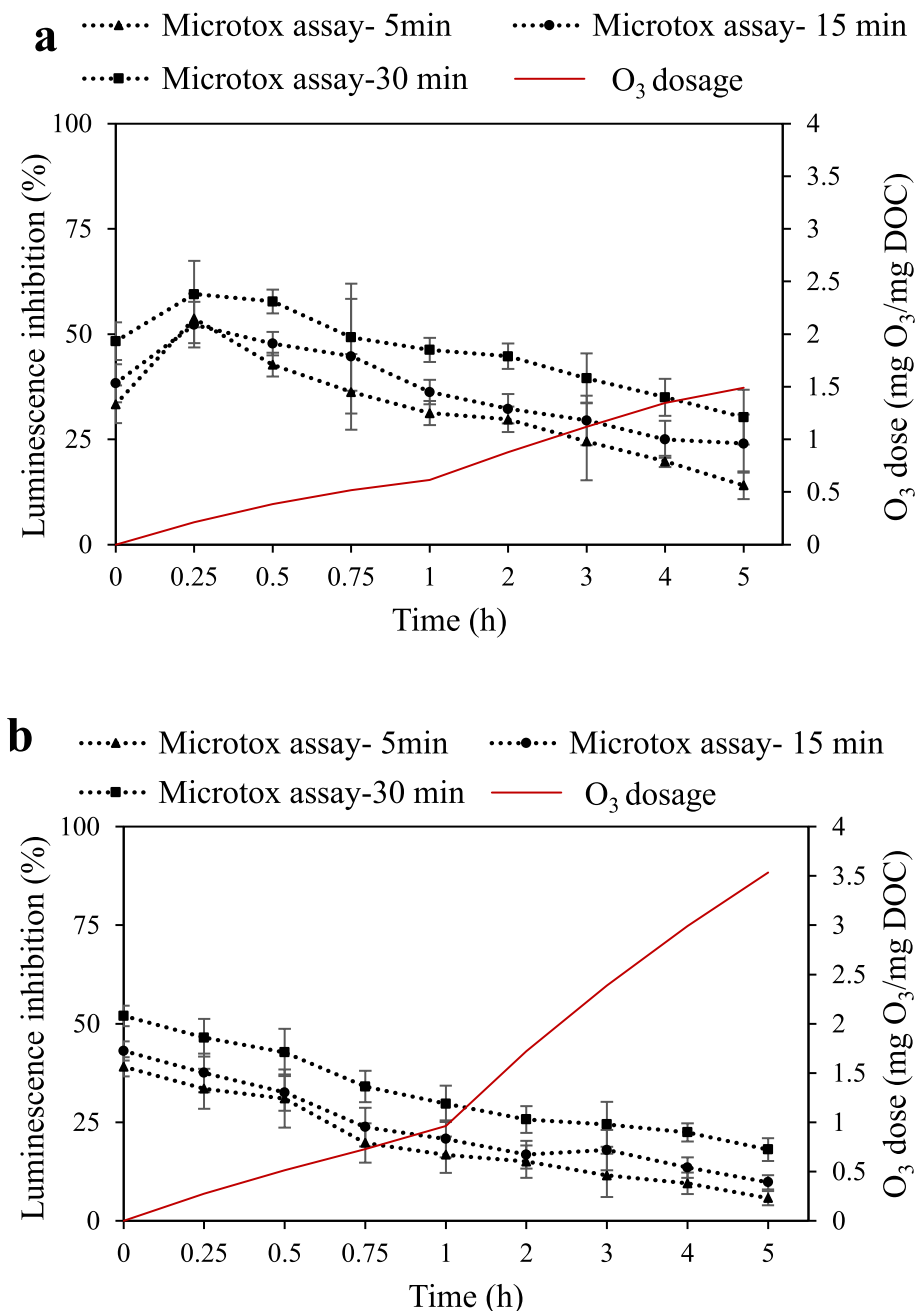


Fig. 6. Comparison the acute toxicity with assay time of 5,15, and 30 min for organism *Vibrio fisheries* in 5 h (a) O₃ and (b) O₃/H₂O₂ treatment in different O₃ dose.

4. Practical applications and future research prospects

This study was to evaluate the feasibility of CECs removal from digestate supernatant with high load of organic and inorganic material and to determine the ozone dose required for CECs removal from digestate supernatant. The digestate from AD plant with manure feed-stock was applied in this research due to the presence of pharmaceutical regarding the animal growth. Ozone-based AOPs showed the effectivity to remove CECs from digestate supernatant, yet, it is recommended to evaluate the method for other types of digestate for instance municipal with wider range and different concentration of CECs. Furthermore, in this research the focus was to determine the ozone demand and assess the general effect of the matrix. More study is needed to comprehend the individual and combined effect of various matrix components on CECs removal, as well as an energy and cost analysis. This will help to better understanding the mechanism and efficiency of CECs removal in ozone-

based treatments.

5. Conclusions

In this study, to remove the most persistent CECs (ibuprofen) from the digestate supernatant, a specific ozone dose of 1.11 mg O₃/mg DOC was required. The matrix composition affects the CECs removal by decreasing the kinetic rate, increasing the ozone consumption, and potentially scavenging the OH $\cdot$. Dissolved organic nitrogen (DON) showed the highest affinity for O₃ consumption. Adding hydrogen peroxide, decreased the O₃ demand to 0.46 mg O₃/mg DOC achieving full CECs removal including ibuprofen. Both O₃ and O₃/H₂O₂ treatment decreased the acute toxicity of digestate supernatant. In general, this study addresses required information regarding ozonation of the digestate supernatant, showing that in spite of the competition effect of the matrix, complete removal of the target CECs was possible in less than

1 h in the digestate supernatant with an ozone dose in the same range as for other (waste-)water matrix. Accordingly, this study suggests that ozonation has the potential to be applied as post-AD treatment for cleaner production.

Credit author statement

Nazanin Moradi: Conceptualization, Methodology, Formal analysis, Investigation, Writing-Original Draft, Visualization, **Carlos Lopez Vazquez:** Conceptualization, Methodology, Writing-Review & Editing, Supervision, **Hector Garcia Hernandez:** Conceptualization, Methodology, Supervision, **Damir Brdjanovic:** Supervision, **Mark C.M. van Loosdrecht:** Supervision, **Francisco Rubio Rincón:** Conceptualization, Methodology, Supervision, Project administration, Funding acquisition.

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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

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envres.2023.116597>.

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