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Application of partial ozonation on tank truck cleaning concentrate and the influence on biodegradability and ecotoxicity: a pilot-scale study

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ABSTRACT

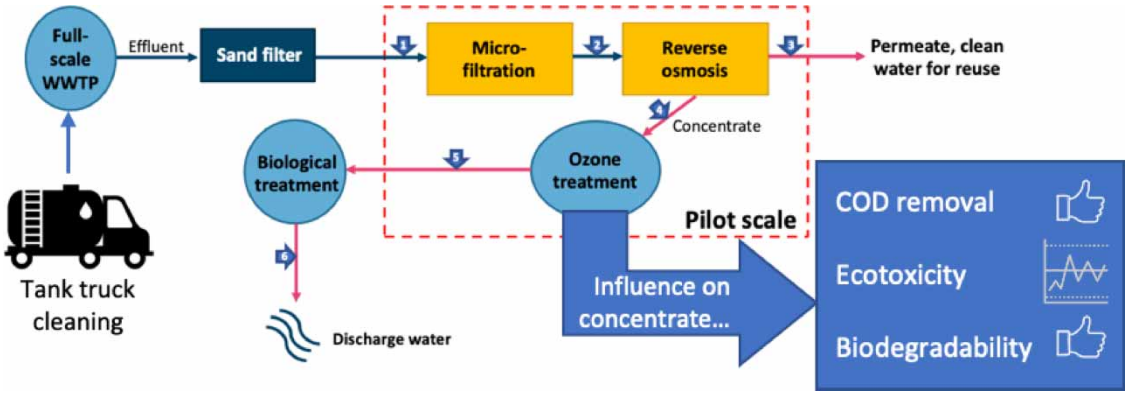
This study investigates the pilot-scale ozone treatment of reverse osmosis concentrate (ROC), originating from variable tank truck cleaning wastewater. The influence of ozonation on short- and long-term biodegradation potential was examined through respirometry and Zahn–Wellens, respectively. Ecotoxicity was also examined for several concentrate batches and ozonation steps. Chemical oxidation through ozone had a beneficial effect on chemical oxygen demand removal, with a removal efficiency up to 56%. Formation of short-term biochemical oxygen demand (BOD_{st}) was induced for several, but not all batches, showing the potential of subsequent biological treatment of ozonated ROC. An increase in the inherent biodegradability through Zahn-Wellens was observed for all tested samples after ozonation, rising to a maximum of 68% after 3 hours of ozonation, highlighting the importance of sludge adaptation. Ecotoxicity, tested with *Artemia franciscana* and the saltwater algae *P. tricornutum,* showed initial decreases in algae inhibition after short ozonation periods. An increase in algae inhibition was, however, seen after prolonged ozonation for all tested ROC samples, pointing to the formation of ecotoxic by-products. *Artemia* showed no significant toxicity effects. When applying biological treatment through Zahn-Wellens, a decrease in ecotoxicity was observed for several samples, likely through biological oxidation of the produced degradation products.

Key words: ecotoxicity, ozone treatment, respirometry, reverse osmosis, tank truck cleaning, Zahn–Wellens

HIGHLIGHTS

- Ozone removed recalcitrant COD from tank truck cleaning reverse osmosis concentrate.
- Short-term and inherent biodegradability increased after extended ozone treatment.
- Ecotoxicity showed initial decrease after ozone, but increased with extended treatment.
- Biological post-treatment was seen as a good way to remove ecotoxic by-products.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Water reclamation has gained much interest in industry nowadays, taking into consideration the water scarcity problems around the globe and regulations limiting water usage. Membrane technologies such as reverse osmosis (RO) treatment are well-known techniques in water reclamation systems, being used both in municipal as well as in industrial wastewater treatment plants. Reverse osmosis produces two important products: permeate which can be reused in industry and reverse osmosis concentrate (ROC), containing mostly organic and inorganic carbon and salts. The composition of this ROC varies largely depending on both the industry where the RO–system is implemented, and the quality of the feed-water (Xiang *et al.* 2019). An important property of ROC is the lack of biodegradability, as described by Deng (2020b) for municipal wastewater ROC. The biodegradability, expressed as biochemical oxygen demand after 5 days of incubation (BOD_5), is commonly very limited with BOD_5/chemical oxygen demand (COD) ratios varying from 0.02 to 0.06, pointing towards a high recalcitrance of the compounds present in ROC.

Wastewater originating from the tank truck cleaning (TTC) industry has a variable composition, due to the different cargos transported in the cleaned tanks, that can vary from chemicals and pharmaceuticals to food. This contributes to the complex nature of TTC wastewater, which is also known to contain ecotoxic compounds (Dries *et al.* 2013; Caluwé *et al.* 2018; Poelmans *et al.* 2020), leading to potential negative effects on the environment when discharged. When RO is conducted on secondary TTC effluent, a very complex and often toxic ROC is formed, as components that were not removed during secondary treatment accumulate in the ROC. De Schepper *et al.* (2009) showed that TTC-generated concentrate contained up to 35 toxic units (TU) for *P. subcapitata*. High toxicity, combined with a higher salinity than typically observed in regular secondary effluents, makes disposal of ROC challenging, stressing the need for further treatment. Several post-treatments have already been examined and proposed for ROC from both municipal and industrial WWTPs for the removal of contaminants. A frequently used post-treatment of ROC is granular activated carbon (GAC), with Dialynas *et al.* (2008) showing dissolved organic carbon (DOC) removal up to 91.3% using GAC for ROC from municipal MBR (membrane bioreactor) effluent. Jamil *et al.* (2020) reported the removal of all DOC fractions in ROC from water reclamation when combining GAC with anion exchange.

More advanced technologies for removing recalcitrant COD and DOC are also gaining interest, such as advanced oxidation processes (AOP). These AOP, including ozonation, UV/H_2O_2, and electrochemical oxidation, have the ability to degrade recalcitrant organic components, removing ecotoxic compounds from industrial effluents (Gottschalk *et al.* 2010; Poelmans *et al.* 2020; Nagels *et al.* 2021). Some research has already been done on the influence of AOP on ROC generated from industrial and municipal secondary effluents (Deng 2020b). Hermosilla *et al.* (2012) investigated the application of photocatalysis, Fenton, and photo-Fenton for the removal of COD in ROC from paper mill effluent. Photo-Fenton achieved the total removal of COD, while conventional Fenton was able to remove up to 80% of COD. Cai *et al.* (2020) examined several AOP, including UV/H_2O_2 and Fenton for COD removal from ROC generated from petroleum industry wastewater, creating removal percentages up to 59%. De Schepper *et al.* (2009) showed ozonation of TTC ROC, removing up to 64% of COD at the optimal ozone dosage, when combined with biological treatment. Ozone reactions can occur in an indirect or direct way, depending on the pH. At a neutral pH, ozone will react both directly and indirectly with compounds, while at

an alkaline pH, the decomposition of ozone to hydroxyl radicals is accelerated. This leads to indirect ozonation, due to a higher reduction potential of the hydroxyl radicals (von Gunten 2003). In some cases, this can lead to higher COD removal/mineralization as described by De Schepper *et al.* (2009), due to the selective nature of direct ozonation.

Next to oxidation, AOP can also be used to increase the biodegradable fraction of wastewater samples. Cai *et al.* (2020) for example showed that BOD_5/COD ratios increased 4.2 to 10 times after fluidized bed reactor (FBR) – Fenton treatment. This opened possibilities for biological post-treatment after AOP, as an increased COD removal of up to 69% was achieved when adding biological activated carbon to the process set up. This combination can decrease the initial cost of the used AOP. De Schepper *et al.* (2009) showed increased biodegradability of TTC ROC for ozonation with measurements of BOD_5, but stated that further COD removal depended on the biodegradation of slow biodegradable components.

The influence of ozonation and AOP, with and without biological post-treatment, on ecotoxicity elimination in ROC is not widely studied, especially for industrial ROC. De Schepper *et al.* (2009) showed ecotoxicity results, with initial increases in *P. subcapitata* toxicity during ozonation of TTC concentrate due to the formation of by-products, followed by a decrease with further ozonation. Biodegradation afterwards further decreased *P. subcapitata* toxicity. Zhou *et al.* (2011) showed decreasing *V. fisheri* toxicity from 62% to 42% when ozonating municipal ROC for 1 hour. Many questions remain, however, about the influence of AOP on ROC regarding inherent biodegradability and ecotoxicity.

The objective of this research was to investigate a pilot-scale setup ozone treatment of ROC originating from TTC secondary effluent. Hereby, the effect on short-term and inherent biodegradability was examined, as well as the influence on acute ecotoxicity after ozonation with and without subsequent inherent biodegradability testing.

2. MATERIALS AND METHODS

2.1. Experimental set up

To assess the influence of ozone treatment on the biodegradability and ecotoxicity of concentrate originating from TTC wastewater, a pilot-scale set up placed on-site at a TTC company was used. The treatment set up is shown in Figure 1. Effluent from the full-scale TTC wastewater treatment plant was collected after sand filtration, but before discharge, at various timepoints in batches of 1 m^3. Each batch was sent through a pilot-scale microfiltration/reverse osmosis (MF/RO) unit, with the produced RO concentrate being transferred to an ozone treatment unit.

MF/RO treatment was conducted with a pilot-scale unit. For RO, a Dow FilmTec SW30 membrane (Dow) was used. Of the treated effluent, 35% left the RO system as concentrate and 65% as permeate, leading to a recovery rate of 0.65 m^3 permeate per m^3 feed. In a consecutive step, the produced concentrate was ozonated in a pilot-scale ozone unit. The unit used corona discharge as the ozone generation mechanism, starting from air with an oxygen concentrator. Ozonation was carried out batchwise in a 185 L tank with continuous recirculation of the concentrate with ozone injection through a venturi valve. Ozone was injected at a gas flow rate of 0.3 $m^3 \cdot h^{-1}$, with a dosage of 100 g $O_3 \cdot m^{-3}$. During the ozonation process, the pH and oxidation–reduction potential (ORP) were constantly monitored. The pH of the concentrate was not changed before or during ozonation.

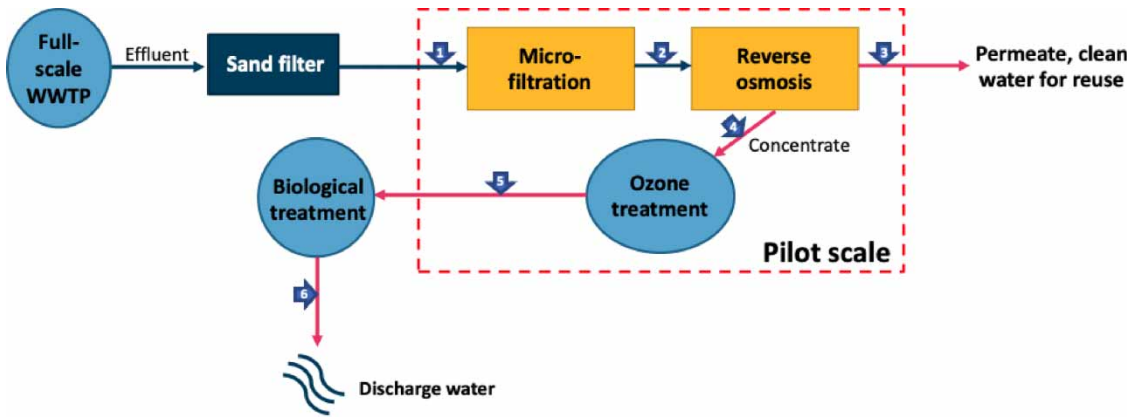


Figure 1 | Overview of the experimental set up. The pilot-scale set up is highlighted, including sampling points (1–6).

Two types of ozone tests were conducted, (1) a preliminary test where two concentrate batches were ozonated to examine the effect on short-term biodegradability, and (2) a definitive test, based on the results of the preliminary test, where ozonation of the concentrate was carried out with the inclusion of inherent biodegradability and ecotoxicity testing. For the preliminary test, two batches of concentrate were ozonated for 2 hours (further on called Batch 1 and Batch 2), with samples being taken before ozonation and after 15 min, 30 min, 60 min, and 120 min of ozone treatment. The measurements that were carried out were COD, soluble COD (sCOD), pH, conductivity (EC), and short-term biodegradability (BOD_{st}). In the definitive test, two batches of concentrate were ozonated for 3 hours (further on called Batch 3 and Batch 4), with samples being taken before ozonation and after 1 h, 2 h, and 3 h of ozonation. The following measurements were carried out for the different concentrate samples in the definitive test: COD, sCOD, pH, EC, acute ecotoxicity, short-term biodegradability (BOD_{st}), and inherent biodegradability (Zahn-Wellens biodegradability test). In this work, the ozone usage is referred to as a specific ozone consumption dose (g consumed O_3 per g DOC_0), as described by Deng (2020a), with the consumed ozone normalized to the initial DOC of the RO concentrate.

2.2. Industrial wastewater

Wastewater originating from a TTC company was used. Effluent from the full-scale WWTP was collected in batches of 1 m^3 and further treated with MF/RO as described in Section 2.1. For the preliminary test only the concentrate samples were characterized, with the concentrate Batch 1 and Batch 2 having a COD value of 805 mg $O_2 \cdot L^{-1}$ and 758 mg $O_2 \cdot L^{-1}$, respectively, before ozonation. As an example of the behavior of COD during the MF/RO-step, the COD for the different filtration steps for Batch 3 is shown in Figure 2. All used concentrate batches showed a pH of 7.

2.3. Effluent and concentrate analysis

The characterization of effluent, concentrate, permeate, and samples for biodegradation tests was done through the analysis of the following parameters: COD, sCOD, pH, and conductivity. COD and sCOD were determined with the HI93754B Medium Range COD test kit (Hanna Instruments, Temse, Belgium). pH and conductivity were measured with Hanna Instruments portable meters HI99141 and HI99301 N, respectively. The DOC of certain samples was measured using a Sievers InnoVox Laboratory Total Organic Carbon Analyzer.

2.4. Acute ecotoxicity

Ecotoxicity measurements were carried out on undiluted effluent and concentrate samples of the definitive test. As stated in Section 2.6 (inherent biodegradability), some samples were diluted (next to the undiluted sample measurement) to comply with the dilution requirements in the Zahn-Wellens biodegradability test. Acute ecotoxicity was measured using a battery of commercially available test kits (Microbiotests, Ghent, Belgium), providing precultured organisms for testing. Due to the high conductivity (>4,000 $\mu S \cdot cm^{-1}$) of the wastewater samples, marine tests were used to eliminate any toxic effects of high salt concentrations: (1) 24 h *Artemia franciscana* brine shrimp mortality test (ASTM 2012) and (2) 72 h *Phaeodactylum tricornutum* marine algal growth inhibition test (ISO 2016). The acute ecotoxicity of the RO permeate was measured using freshwater tests: (1) 48 h *Daphnia magna* water flea immobilization test (OECD 2004) and (2) 72 h *Pseudokirchneriella subcapitata* algal growth inhibition test (OECD 2011).

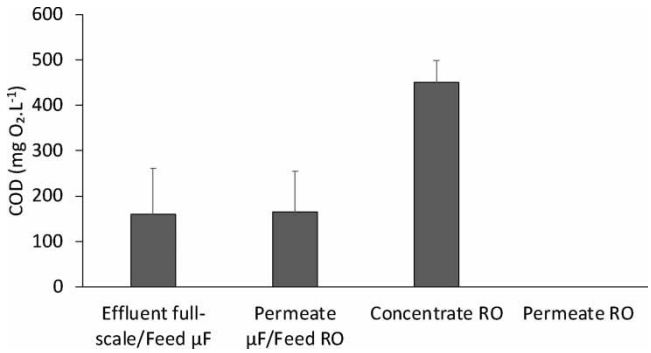


Figure 2 | COD for wastewater Batch 3, used in the definitive test. Error bars represent the standard deviation from the average COD.

2.5. Short-term biodegradability (BOD_{st})

The short-term biochemical oxygen demand (BOD_{st}) is a measurement for the short-term biodegradability of the available COD in wastewater, it was determined through respirometry experiments. In these experiments, the evolution of the oxygen uptake rate (OUR) of the activated sludge is monitored after addition of the tested sample. For each batch (both screening and definitive test), one test was set up for each available sample of concentrate and ozonated concentrate. The respirometry tests were conducted in 400 mL glass beakers. The set ups were equipped with a Vernier Optical DO probe (Vernier, USA) for oxygen measurement. Aeration was done between two setpoints (2–4 mg $O_2 \cdot L^{-1}$) and provided by Aqua-Forte aeration pumps with diffusion into the liquid with stone air balls. Logging of the oxygen concentration and subsequent calculation of OUR was done through LabView™-software (National Instruments, USA). During each test, 200 mL of activated sludge originating from a full-scale WWTP of a TTC company was used, with an addition of 100 mL of concentrate sample.

Each respirometry test ran for 72 h. Every 24 h, the added concentrate sample was refreshed through a settling and discharge procedure. After settling, 100 mL of supernatant was discharged followed by the addition of 100 mL fresh concentrate sample. This set up resulted in three respirograms (OUR vs. time graphs) for each sample, further called run 1, run 2, and run 3, which were used for the BOD_{st} calculation for the sample. The BOD_{st} for each run was calculated according to Equation (1), where $BOD_{st,sample}$ is the short-term biological oxygen demand of the sample in mg $O_2 \cdot L^{-1}$, V_{sludge} is the volume of the sludge (L), V_{sample} is the volume of the added sample (L), and OUR_{exo} is the exogenous oxygen uptake rate (mg $O_2 \cdot L^{-1} \cdot h^{-1}$). The area beneath the OUR graph was determined, giving the amount of oxygen used to biologically degrade the available substrate. In the results section, only BOD_{st} results for run 3 are shown and compared:

$$BOD_{st,sample} = \frac{V_{sludge} + V_{sample}}{V_{sample}} \times \int_{t_0}^{t_n} OUR_{exo}\,(t) \times dt \tag{1}$$

2.6. Inherent biodegradability

The longer-term or inherent biodegradability of non-ozonated and ozonated concentrate samples of the definitive test was determined using the Zahn-Wellens biodegradability test according to OECD guideline 302B (OECD 1992). Activated sludge from a full-scale WWTP of a TTC company was washed with mineral medium (composition according to OECD 302B) to remove any contaminants. Glass bottles were filled with a 1 L mixture of activated sludge and sample with known concentrations, with the addition of mineral medium for dilution when necessary. A blank test with sludge and mineral medium, and a reference test with sludge and a reference compound were also conducted. Samples that were tested for Batch 3 for inherent biodegradability were (1) RO concentrate before ozonation, (2) RO concentrate after 2 h ozonation, and (3) RO concentrate after 3 h ozonation; for Batch 4 the RO concentrate after 1 h ozonation was also tested.

The sludge/sample mixtures were aerated and mixed for 28 days at a constant temperature of 22.5 °C. During the 28 day testing period, samples were taken for sCOD analysis after 3 hours (on the first day) and after 3, 7, 10, 13, 17, 19, 24, and 28 days. At the end of the test, aeration and mixing were stopped and the supernatant of all test bottles was collected for acute ecotoxicity measurements. The inherent biodegradability was calculated as percentage sCOD degradation after 28 days according to OECD 302B using Equation (2) where D_t is the percentage degradation at time t (%), C_A is the concentration of COD in the test suspension 3 h after the start of the test (mg·L^{-1}), C_{BA} is the concentration of COD in the blanks 3 h after the start of the test (mg·L^{-1}), C_B is the concentration of COD in the blanks at time t (mg·L^{-1}), and C_t is the concentration of COD in the test suspension at time t (mg·L^{-1}):

$$D_t = \left(1 - \frac{C_t - C_B}{C_A - C_{BA}}\right) \times 100 \tag{2}$$

3. RESULTS AND DISCUSSION

3.1. Preliminary test on short-term biodegradability

Ozonation was conducted on concentrate Batches 1 and 2 (see Section 2.1) in a preliminary test, to examine the effect of ozone on the short-term biodegradability. COD degradation and short-term biodegradability were monitored during the

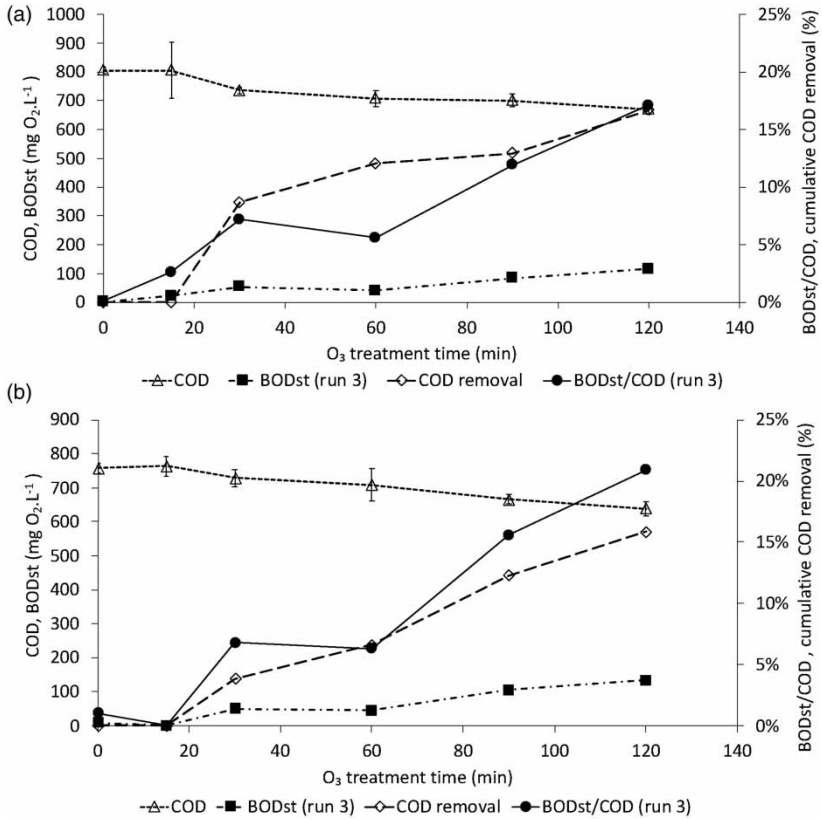


Figure 3 | COD, BOD_{st}, COD removal and BOD_{st}/COD results for (a) Batch 1 and (b) Batch 2 for different ozonation times. Error bars represent standard deviations for average COD.

test. Figure 3 shows the results of the respirometry tests, and the COD and BOD_{st} measurements (for run 3). Initial COD values for Batches 1 and 2 were 805 mg $O_2 \cdot L^{-1}$ and 758 mg $O_2 \cdot L^{-1}$, respectively. During 2 hours of ozonation, an increasing BOD_{st} was observed for Batches 1 (Figure 3(a)) and Batch 2 (Figure 3(b)). The maximum BOD_{st} value was reached for both Batches 1 and 2 after 120 min. ozonation, with run 3 resulting in 114 and 133 mg $O_2 \cdot L^{-1}$, respectively.

The BOD_{st} values were compared to the COD values of the samples. These results, including the BOD_{st}/COD ratio percentages and COD degradation, are shown in Figure 3. Only 17 and 16% of the initial COD was removed for Batches 1 and 2, respectively, after 2 hours of ozonation. Batches 1 and 2 showed a specific ozone consumption dose of 0.28 and 0.30 $gO_3 \cdot gDOC_0^{-1}$, respectively. Compared to Justo *et al.* (2013), where 37% of COD was removed in municipal water reclamation RO brine with an ozone dose of 6.93 $gO_3 \cdot gTOC^{-1}$, this shows that the ozone was used more effectively for direct COD removal in this study. The highest BOD_{st}/COD ratio was achieved after a 2-hour ozone treatment of the concentrate. This indicated that despite low COD removal, a relatively high biodegradable fraction could be achieved, which is desirable for potential biological post-treatment of the ozonated concentrate. This is comparable to results achieved by De Schepper *et al.* (2009), where an increase in the biodegradable fraction was seen when ozonating TTC concentrate at pH 7.5, while having lower total COD removal (without biological treatment afterwards) compared to higher pH setpoints. At a neutral pH, ozone reactions will occur very selectively, with less effective mineralization.

3.2. Definitive test: short-term biodegradability

Based on the results of the preliminary test described above, an ozone treatment step of 3 h was implemented for the ozonation of Batches 3 and 4. During these 3 h, concentrate samples were taken at the start and after 1 (only Batch 4), 2, and 3 h of ozonation. Figure 4 shows the achieved short-term biodegradability, including COD removal, BOD_{st} (run 3), and BOD_{st}/COD (run 3) values. Batch 3 (Figure 4(a)) showed similar results as in the preliminary test, with a steady increase in BOD_{st} to 124 mg $O_2 \cdot L^{-1}$ during run 3. For Batch 4 (Figure 4(b)) an opposite result was obtained, as none of the samples showed short-term

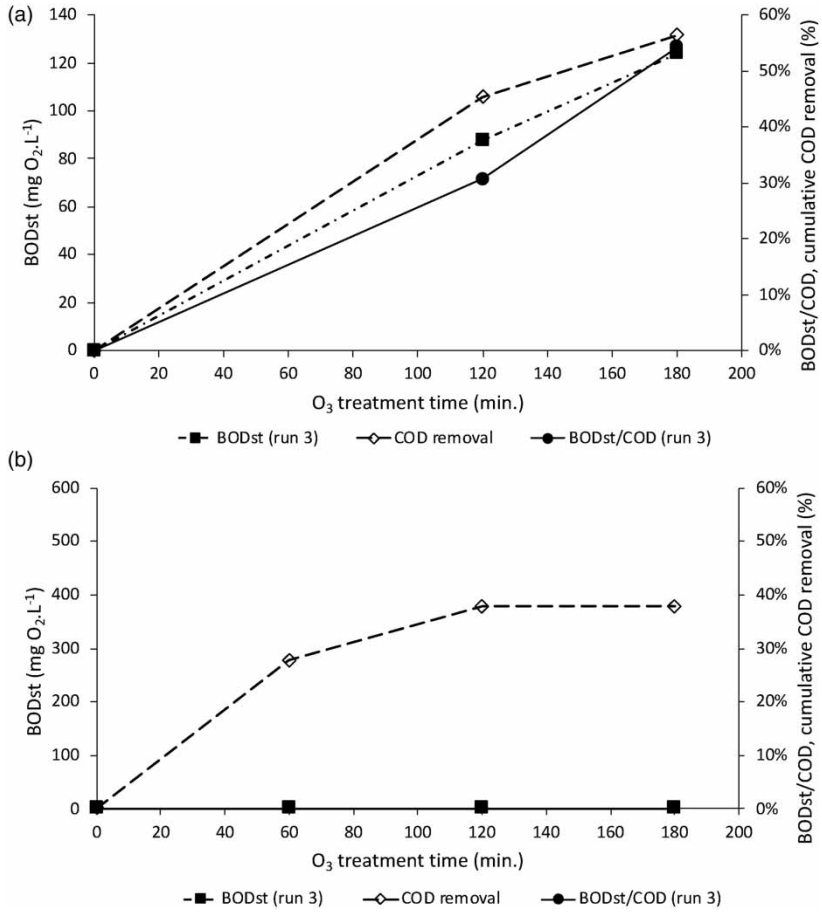


Figure 4 | BOD_{st}, COD removal and BOD_{st}/COD results for (a) Batch 3 and (b) Batch 4 for different ozonation times.

biodegradability during the respirometry tests. A possible explanation could be the full mineralization of COD without formation of biodegradable components. The composition of the concentrate, coming from TTC wastewater with a high variability, probably played a role. Depending on the composition and especially the complexity of the compounds in the sample, a better removal can be achieved. This is a phenomenon that was also encountered in previous research (Poelmans *et al.* 2020).

Regarding COD removal and BOD_{st}/COD ratios (Figure 4), a 56% removal of COD was recorded for Batch 3, and 37% for Batch 4 after 3 hours of ozonation. Specific ozone consumptions of 0.52 and 0.76 $gO_3 \cdot g\ DOC_0^{-1}$ respectively were observed, showing similar COD removal percentages to Justo *et al.* (2013), but with 6.93 $gO_3 \cdot gTOC^{-1}$ dosed, the ozonation was more effective in removing COD in the present study. For Batch 4, the maximum COD removal of 37% was already seen after 2 hours of ozonation. When comparing the COD removal after 2 hours with the results of the preliminary test, higher COD removals of 45 and 37% were achieved for Batches 3 and 4, respectively. Similar removals were seen by Loh *et al.* (2021), where a 42% removal efficiency was observed for 1 h microbubble ozonation of ROC originating from a reclamation facility located in a petrochemical wastewater treatment plant. When using standard ozonation, a 30% removal was observed, closer to observations made for Batch 4 in this study. Significant decreases in COD were also observed in the study of De Schepper *et al.* (2009) for ozonation of TTC concentrate, as described in Section 3.1, at two pH setpoints (7.5 and 11.5). COD was highly mineralized when using pH 11.5, compared to a clear formation of biodegradable COD at a neutral pH. The latter was also observed for Batch 3 in the present study combined with a relatively high COD removal of 56%, using direct ozonation.

The higher COD removal for Batch 3, compared to Batches 1 and 2, and probable mineralization after 2 hours did not result in a decrease of the BOD_{st}/COD ratio. A BOD_{st}/COD ratio of 30% was achieved after 2 hours of ozonation for

Batch 3. The BOD_{st}/COD ratios showed a further increase with longer ozonation for Batch 3, increasing up to 54.3% for run 3, having a high biodegradable fraction. In contrast, no biodegradability change could be observed for Batch 4. In comparison, Loh *et al.* (2021) observed increases in BOD_5/COD from 0.042 to 0.216.

3.3. Definitive test: inherent biodegradability

To get a clear view on the influence of ozone on concentrate biodegradability, the inherent biodegradability of the samples from Batches 3 and 4 was tested in the Zahn-Wellens inherent biodegradability test, running for 28 days. The percentual COD degradation at the end of the test is shown in Figure 5. The reference compound used, monoethylene glycol (MEG), was completely degraded during the first 10 days, in line with validation criteria for the Zahn-Wellens test. 2 h ozonated concentrate from Batch 3 showed an increase in COD degradation after 28 days from 25 to 55% of the initial COD. No further improvement was observed after 3 h of ozonation. The increase in inherent biodegradability of Batch 3 after 2 h of ozonation was comparable to the trend observed for short-term biodegradability in the respirometry tests (Figure 4).

For Batch 4 the opposite of the short-term biodegradability tests was achieved, with a clear increase in the inherent biodegradability with a longer ozone treatment time. A maximum could be found at 3 hours of ozonation with a COD degradation of 64%. The increase of inherent biodegradability during ozonation was in line with results obtained by Gomes *et al.* (2021) for denitrified landfill leachate, with an increasing biodegradability from 11 to 73% after ozonation. According to Płuciennik-Koropczuk & Myszograj (2018) for the samples to be classified as well-biodegradable, 70% removal should be obtained, with no samples in this study ultimately reaching this threshold. The presence of inherent biodegradability and absence of short-term biodegradability observed for Batch 4, still highlight the importance of adaptation of the sludge to different substrate compositions.

3.4. Definitive test: ecotoxicity after ozone treatment and biodegradation

Acute ecotoxicity was measured for the samples of Batches 3 and 4 before and after ozonation, including the starting effluent. Permeate toxicity towards *D. magna* and *P. subcapitata* was 0% for both Batches 3 and 4. The *P. tricornutum* algal growth ecotoxicity effect of the secondary effluent originating from the full-scale WWTP was 85 and 64% for Batches 3 and 4, respectively (Figure 6(a) and 6(b)). For *Artemia franciscana,* no toxic effects were observed in the secondary effluent, and it remained negligible during ozonation. The toxicity effect towards *P. tricornutum* increased to 100% in RO concentrate in both cases, due to the concentration of recalcitrant and toxic components.

2 h of ozonation of the Batch 3 concentrate resulted in an almost complete removal of the algal growth inhibition (Figure 6(a)). However, extended ozonation led to increased inhibition of algal growth to 52%, most likely due to the

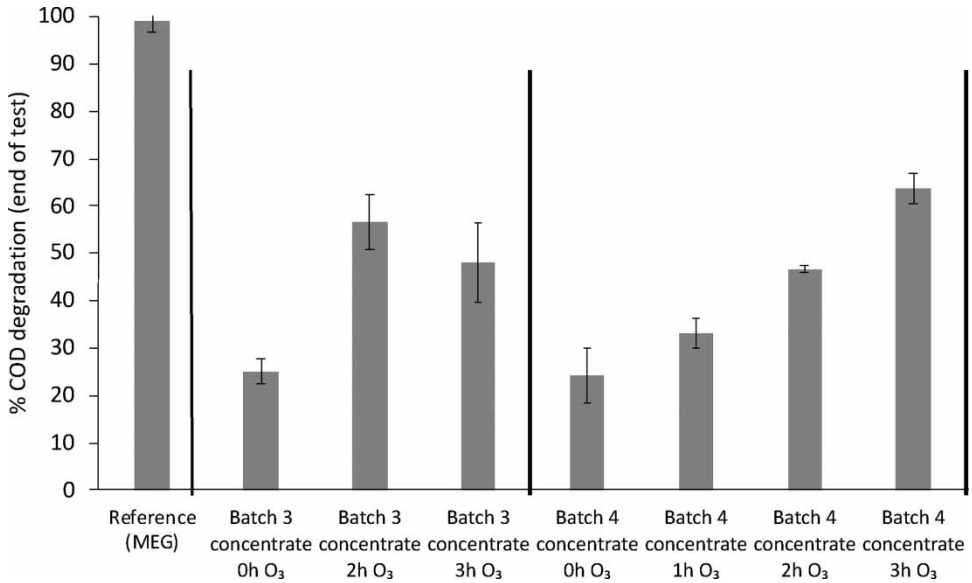


Figure 5 | Final degradation percentages for concentrate samples of the 28 days Zahn-Wellens test for Batch 3 and Batch 4. Error bars represent standard deviations.

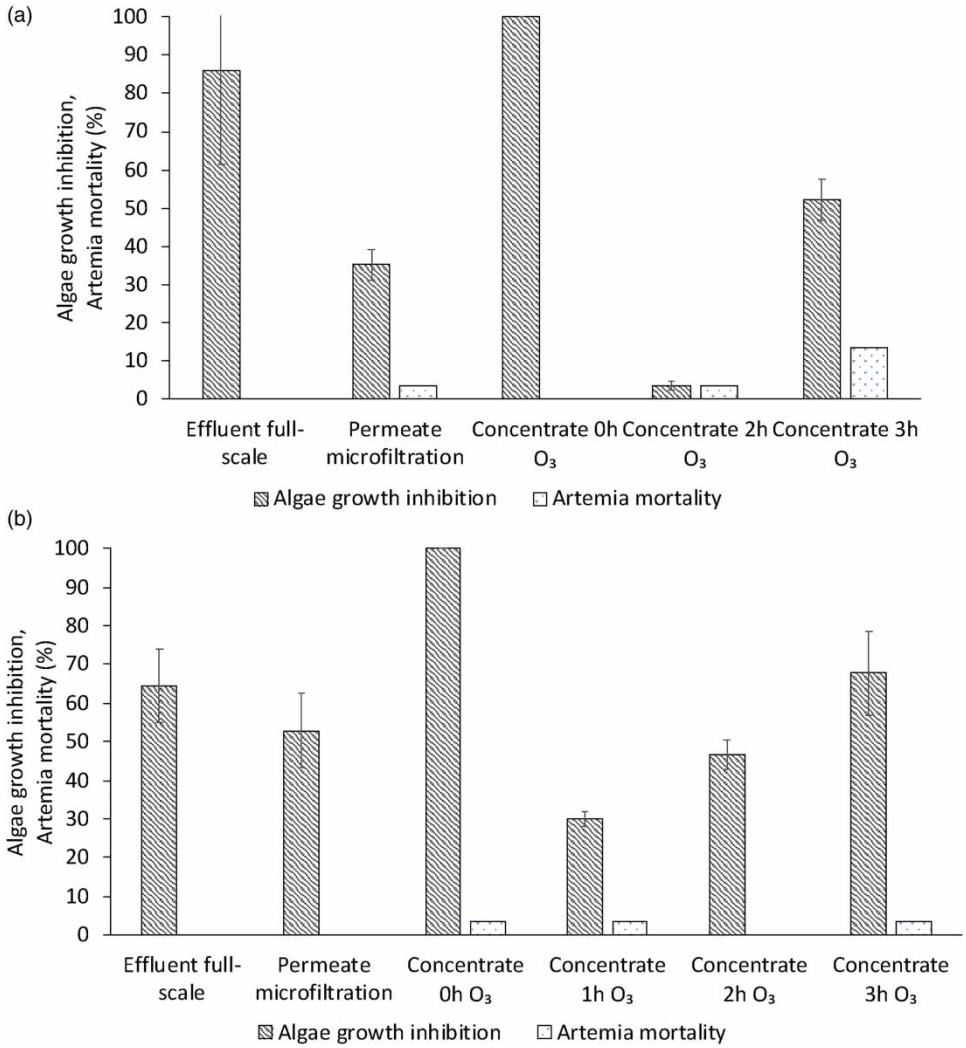


Figure 6 | Acute ecotoxicity for (a) effluent and concentrate samples from Batch 3 and (b) effluent and concentrate samples from Batch 4. Error bars represent standard deviations.

formation of toxic degradation products. Batch 4 showed similar initial results, with toxicity towards *P. tricornutum* in the algal growth inhibition test initially decreasing after 1 hour of ozone treatment. An increase was seen when ozonation was extended (Figure 6(b)). This increase is a phenomenon also observed by De Schepper *et al.* (2009), where algal inhibition reaches a minimum of 13–14 TU (starting point = 32 TU) when ozonating TTC ROC at both neutral and alkaline pH during certain ozonation periods. The toxicity relapsed again for the alkaline pH when ozonating for a longer period when compared to the initial values. Toxicity towards *D. magna* was not significant, comparable to *Artemia* results in the present study, with any toxicity being prescribed to the high conductivity of the concentrate. Ozonation is therefore a good strategy to remove ecotoxicity from TTC concentrate, but the formation of toxic by-products is not negligible. Weng *et al.* (2018) observed no change in toxicity for algae inhibition using *E. gracilis* when ozonating the ROC of a municipal WWTP, despite a clear TOC decrease of almost 50%. This confirms the observations made in this study, a decrease in COD does not necessarily mean that the ecotoxicological standards are met, the measurement of ecotoxicity therefore remains important.

Ecotoxicity measurements were carried out at the end of the Zahn-Wellens inherent biodegradability test to evaluate the influence of biological post-treatment on ecotoxicity. For comparison, the (ozonated) concentrate samples were diluted to match the dilution applied in the Zahn-Wellens test (Figure 7(a)).

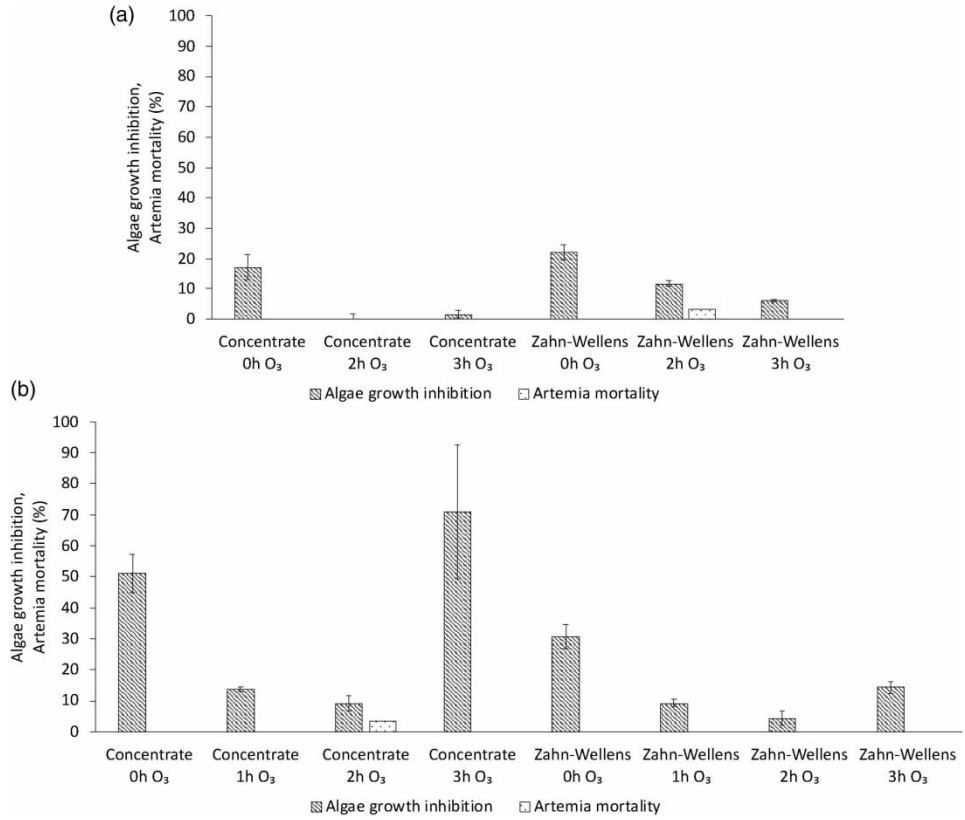


Figure 7 | Ecotoxicity of (ozonated) concentrate before and after Zahn-Wellens inherent biodegradability testing for (a) Batch 3 and (b) Batch 4. Dilutions applied: 2x for Batch 3, 0.11x for Batch 4. Error bars represent standard deviations.

Ecotoxicity results for Batch 3 (Figure 7(a)) showed a slight increase (<20%) in toxicity towards algal growth for all ozonation durations. For Batch 4 (Figure 7(b)), a small decrease in algal growth inhibition was observed when the Zahn-Wellens test was carried out, suggesting the biodegradation of toxic ozonation products from the concentrate matrix. A decrease in algal growth inhibition of over 50% was observed for the concentrate sample after 3 h of ozonation. When comparing the ecotoxicity results in the present study to results obtained by De Schepper *et al.* (2009), no significant toxicity difference for the algae test was observed when implementing short-term biological treatment at a neutral pH. On the other hand, at an alkaline pH, a significant decrease in toxicity was observed. Toxicity towards *D. magna* was not significant, which is comparable to the *Artemia* results in the present study. The observations made in the present study and previous studies indicate that a biological post-treatment of ozonated TTC concentrate can have a positive effect on further ecotoxicity removal.

4. CONCLUSIONS

In this study, ozonation was found to be a feasible option to remove recalcitrant COD from ROC, originating from TTC effluent. COD removal up to 56% was achieved during pilot-scale ozone treatment, with increases in biodegradability up to a BOD_{st}/COD ratio of 54.3%. High inherent biodegradability values could be achieved for ozonated ROC samples using the Zahn-Wellens test. This favors the use of a subsequent biological treatment to further decrease COD. The impact of ozonation on ecotoxicity removal is quite inconsistent due to the complexity and variability of the TTC effluent. This variability of ROC TTC wastewater makes for a challenging future approach, as every effluent can act differently through ozonation. Biological treatment (e.g. aerated sand filter or activated carbon filter) of ozonated TTC ROC is therefore useful to reduce ecotoxicity. Future research should be focused on investigating different treatment combinations. In conclusion, the proposed treatment in this study (ozonation followed by biological treatment) provides a useful solution for ROC streams of TTC effluent, making water reuse possible.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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