Removal of Sulfadiazine from Simulated Industrial Wastewater by a Membrane Bioreactor and Ozonation

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Abstract

Ozonation can be used as a polishing treatment for degrading low-concentration pharmaceutical compounds recalcitrant to biological treatment, when large amounts of biodegradable organics have been previously removed by biological processes. Nevertheless, a systematic investigation has not yet been carried out for the coupled MBR+O_3 process through an experimental design approach. Thereby, the purpose of this study is to evaluate the performance of different processes (membrane bioreactor-MBR, ozonation; and integrated MBR+O_3) for removing the antibiotic sulfadiazine (SDZ) from a synthetic wastewater matrix of industrial interest. The MBR behavior was monitored over seven months for different parameters (pH, temperature, permeate flow, transmembrane pressure, biological oxygen demand-BOD_5, chemical oxygen demand-COD, total organic carbon-TOC, solids, and SDZ concentration). Additionally, the amount of SDZ sorbed onto the sludge was characterized, an issue which is scarcely addressed in most research works. Ozonation experiments were conducted in batch mode in a 2-L glass reactor provided with openings for gas flow. For the MBR+O_3 process, the effects of gas flow rate (0.1-1.5 L min^{-1}) and inlet ozone concentration (4-12 mg L^{-1}) on SDZ removal from the MBR permeate were systematically assessed using a Doehlert experimental design and response surface methodology. The results indicated that the MBR system showed good performance regarding organic matter removal efficiency, evaluated in terms of BOD_5 (91.5%), COD (93.1%) and TOC (96.3%). In contrast, SDZ was partially removed (33%) by the MBR; in that case, the results indicated that the antibiotic was moderately removed with the sludge and partially biodegraded. In turn, the MBR+O_3 system showed excellent performance for removing SDZ (100%), TOC (97%), BOD_5 (94%) and COD (97%). The statistical analysis confirmed that the influence of ozone gas flow rate upon the SDZ removal rate was more important than that exhibited by inlet ozone concentration. Therefore, coupling MBR and ozone can be considered a promising alternative for point source treatment of antibiotic production wastewater.

Keywords

Membrane Bioreactor (MBR); Ozonation (O_3); Integrated process; Sulfadiazine; Modified polymeric membranes; Doehlert uniform array

1. Introduction

Antibiotics are an important class of pharmaceuticals used for treating and preventing infectious diseases caused by bacteria in humans and animals. These compounds have been detected in various aqueous environments, such as surface, ground and even drinking water, causing growing concerns regarding the selection of antibiotic resistant microorganisms (Novo et al. 2013). High antibiotic concentrations, some of the order of milligrams per liter, have been found in the effluents from drug manufacturing facilities (Larsson et al. 2007). Among them, sulfonamides are used worldwide, and their elimination during conventional wastewater treatment processes has been found to be rather low (Dolar et al. 2012, Zhang et al. 2015).

Membrane bioreactors (MBR), which combine biodegradation by activated sludge with solid-liquid separation using membrane filtration in a single step, have become a widespread wastewater treatment alternative in the last decade (Krzeminski et al. 2017). Their main advantages are the small size, limited sludge production and high-quality effluents produced, which are particularly suitable for subsequent treatment (Vo et al. 2019). Previous works have reported sulfonamides removal in MBR systems via sorption and biodegradation (Galan et al. 2012, Tambosi et al. 2010, Yu et al. 2018). Nevertheless, removal yields are not yet satisfactory, varying in the range 64-90% for sulfadiazine and sulfamethoxazole, operating at different hydraulic retention times (HRT $\geq$ 12 h) (Yu et al. 2018). Despite this issue, micropollutants, removal efficiencies can be improved by combining membrane bioreactors to an oxidation process, such as ozonation (Echevarria et al. 2019).

During ozonation, pollutants are oxidized either by direct reaction with aqueous ozone or indirectly by hydroxyl radicals, which are generated as a result of O_3 decomposition in alkaline medium (Hansen et al. 2016). Ozonation has shown to be effective for antibiotics oxidation (Guo et al. 2016, Urbano et al. 2017), and its use as a polishing technology in wastewater treatment plants (WWTP) is widely accepted (Hansen et al. 2016). For example, Nielsen et al. (2013) studied the removal of bacteria and active pharmaceutical ingredients, among which different sulfonamides (sulfadiazine, sulfamethazine, sulfamethizole, sulfamethoxazole) by MBR combined with O_3, O_3/H_2O_2, powdered activated carbon (PAC) or chlorine dioxide (ClO_2). Their results suggested the MBR+PAC and MBR+O_3 as the most efficient and cost-efficient systems, respectively. Nevertheless, a systematic investigation has not yet been carried out for the coupled MBR+O_3 process through an experimental design approach.

Given that, the present work aims at investigating the removal of the antibiotic sulfadiazine (SDZ) in a synthetic wastewater matrix, on pilot and bench scales, through a membrane bioreactor (MBR) as well as the integrated MBR+O_3 technology. The behavior of the MBR was monitored over seven months for different parameters (pH, temperature, permeate flow, transmembrane pressure, BOD_5, COD, TOC, solids, and SDZ concentration). Moreover, analyses of SDZ sorbed onto the sludge were performed, an issue which is not usually addressed in most research works (Dolar et al. 2012). Finally, for the MBR+O_3 process, the effects of gas flow rate and inlet gaseous ozone concentration upon SDZ removal from the

MBR permeate were systematically assessed using a Doehlert experimental design and response surface methodology.

2. Materials and methods

2.1. Reagents

SDZ (99%), obtained from Sigma-Aldrich, was employed as a model antibiotic of emerging concern. Methanol (HPLC quality) and acetic acid (80% v/v) used as solvents for high pressure liquid chromatography (HPLC), were acquired from Panreac and Scharlau, respectively.

The MBR system was fed with synthetic wastewater, the composition of which is reported in Table SM_1 (Biosic et al. 2017, Xu et al. 2014). Micro-nutrients were dosed according to the expected requirement for biomass growth. All the reagents were diluted in tap water.

2.2. MBR setup

The 144-L operating volume MBR pilot plant used herein was equipped with a module of 21 modified polyethersulfone submerged ultrafiltration membranes (filtering area 0.071 m^2), with nominal pore size of 0.01 μm and dimensions 22.5 × 31.5 × 0.5 cm. The membranes were synthetized at the International Reference Center on Water Reuse (IRCWR), and modified with the addition of bentonite and montmorillonite nanoparticles. A simplified scheme and a photograph of the MBR system are depicted in Figure SM_1.

The MBR was fed with synthetic wastewater (Table SM_1) and operated under continuous aeration (15 L min^{-1}) and hydraulic retention time (HRT) of 12 h. The sludge retention time (SRT) in the MBR was maintained at 31 days via sludge withdrawal. A sulfadiazine (SDZ) stock solution (50 mg L^{-1}) was mixed with the liquid fed to the reactor to obtain an inlet SDZ concentration of about 10 mg L^{-1}, a value similar to those reported for antibiotics in effluents from pharmaceutical formulation facilities (Fick et al. 2009). Na_2CO_3 (0.5 mol L^{-1}) was added to maintain the pH above 6. The system operated at room temperature, measured with a Naka type 8611 sensor. The permeate flow rate was measured with a Burket type 8611 sensor.

During MBR operation, suction (8 min) and relaxation (2 min) cycles were alternated to reduce membrane fouling. The transmembrane pressure (TMP) was measured using a pressure sensor (Gulton, GTO 1000 model). Once the TMP reached approximately 20 kPa, off-line backwashing, using a 200-mg L^{-1} sodium hypochlorite solution was carried out to remove the bio cake formed on the membranes surface. In addition, to maintain a constant liquid level in the MBR reactor and to prevent membranes exposure to air, the peristaltic pumps (Etatron, E. Co.pH model) used to deliver the synthetic wastewater to the reactor and to remove the permeate were connected to a liquid level sensor.

The real-time monitoring of the MBR operation parameters (pH, temperature, permeate flow, TMP) was performed using a Novus data logger. Samples were taken regularly from the feeding and permeate, and were analyzed for different parameters (biological oxygen demand-BOD_5, chemical oxygen demand-COD, total organic carbon-TOC, volatile suspended solids-VSS, total suspended solids-TSS, and SDZ concentration), according to the protocols of the Standard Methods for the Examination of Water and Wastewater (APHA, 2012). Finally, selected sludge samples were analyzed for SDZ concentration.

2.3. Ozonation setup

Ozonation experiments were conducted in batch mode with gas feed, in a 2-L jacketed glass reactor with continuous magnetic stirring (300 rpm), provided with openings for gas inlet and outlet, sampling collection and venting. Ozone was produced using an ozone generator (Multivacuo, MV-06/220 model) fed with pure oxygen. The oxygen-ozone gas mixture was continuously fed to the reactor at a fixed flow rate through a cylindrical porous ceramic diffuser (5 cm long and 1.8 cm in diameter) located at the reactor bottom. The O_3 concentration in gas was monitored spectrophotometrically at 254 nm using a 1-cm quartz flow cuvette. The temperature of the reactor content was kept at 25 ± 1 °C by using a thermostatic bath (Julabo, ME F25 model). Exactly 0.5 L of MBR permeate was used in the ozonation experiments.

An experimental Doehlert uniform array design (Ferreira et al. 2004) for two variables (gas flow rate, 0.1-1.5 L min^{-1}; and ozone inlet concentration, 4-12 mg L^{-1}) was used to study SDZ removal from the MBR permeate by ozonation (Table 1). The pH and SDZ concentration of the permeate used in these experiments were (6.28 ± 0.19) and (7.68 ± 0.19) mg L^{-1}, respectively. The permeate used in all runs was collected from the MBR on the same day, to avoid fluctuations in the initial antibiotic concentration. An additional ozonation experiment (0.8 L min^{-1} gas flow rate; 8 mg O_3 L^{-1}) of the SDZ-containing synthetic wastewater (pH 5.63; $[SDZ]_0$ = (10.60 ± 0.07) mg L^{-1}) was carried out for providing a better comparison of the performances of sole MBR, ozonation, and MBR+O_3. In any case, 3-mL samples were withdrawn from the liquid and analyzed for SDZ concentration by HPLC. The initial and final TOC concentrations were also measured.

Table 1. Doehlert design array for two factors. Variables X_1 and X_2 represent the coded values of gas flow rate and inlet O_3 concentration, respectively. Experimental conditions: $[SDZ]_0$= (7.68 ± 0.19) mg L^{-1}, pH_0 = (6.28 ± 0.19), 25 °C.

Exp. number	**Coded values**		**Uncoded factors**		**Response variable**
	X_1	X_2	**Gas flow rate (L min^{-1})**	**Inlet O_3 concentration (mg L^{-1})**	k_{SDZ} **(min^{-1})**
1	0	0	0.8	8.0	1.04
1'	0	0	0.8	8.0	1.12
1"	0	0	0.8	8.0	1.13
2	1	0	1.5	8.0	1.62
3	0.5	0.866	1.15	12.0	1.83
4	–1	0	0.1	8.0	0.85
5	–0.5	–0.866	0.45	4.0	0.82
6	0.5	–0.866	1.15	4.0	0.70
7	–0.5	0.866	0.45	12.0	0.82

The response of the Doehlert design was the pseudo first-order specific SDZ degradation rate (k_{SDZ}, min^{-1}). Fitting of the response surface model (Equation 1) to experimental data was carried out using the software Statgraphics Centurion XVI. The standard-deviation of the response variable (k_{SDZ}) was 0.05 min^{-1}, based on the triplicates of the central point of the experimental design.

$$k_{SDZ}(\text{min}^{-1}) = a_0 + a_1X_1 + a_2X_2 + a_{11}\,X_1^2 + a_{22}X_2^2 + a_{12}X_1X_2 \qquad (1)$$

2.4. Analytical methods

An HPLC system (Shimadzu, LC20 model) equipped with a UV/VIS diode array detector (SPD-20A model) and a RP18 column (Super Sphere 100 model, 250 mm × 4.6 mm; 5 μm) was used to follow SDZ concentration over time. The following conditions were used: 80% aqueous acetic acid 1% (v/v) + 20% methanol, isocratic. The temperature, injected volume, and mobile phase flow rate were 40 °C, 100 µL, and 1 mL min^{-1}, respectively. SDZ absorption was measured at 266 nm; under these conditions, the SDZ retention time was about 6.4 min. The detection and quantification limits were 3.2 and 9.7 µg L^{-1}, respectively.

The TOC concentration of the selected samples was measured using a carbon analyzer (Shimadzu, TOC-L).

The extraction and determination of SDZ in sludge samples were performed using the QuEChERS method, adapted from Cerqueira et al. (2014). Briefly, SDZ extraction was achieved by placing 10 g of sludge into a 50-mL Falcon tube. Subsequently, 10 mL of acetonitrile acidified with 100 µL of acetic acid were simultaneously added to the tube, which was then hand-shaken for 15 s; after that, the tube was shaken vigorously in a vortex for 1 min. Afterwards, partitioning salts (4 g $MgSO_4$, 1 g NaCl) were added to the tube, which was vigorously shaken, first by hand (15 s) and then using the vortex (1 min). After that, the sample was centrifuged at 3000 rpm for 5 min. The supernatant was taken, filtered and injected into the HPLC system.

3. Results and discussion

3.1. MBR treatment

The MBR pilot-plant operation comprised three phases, detailed in the following sections. Table 2 presents the operating conditions of the MBR system in each phase. Fig. 1 shows the time profiles of TMP, pH, permeate flow rate (Q) and temperature during phases II and III.

Phase I: Sludge acclimatization

Before the MBR operation, 60 L of activated sludge were taken from another biological reactor in operation at IRCWR, and used as inoculum. The sludge sample was mixed with sewage in an 85-L aerated tank, operating in batch. Synthetic wastewater containing no SDZ was then gradually introduced into the tank. The sludge was acclimatized for 50 days until the concentration of the total suspended solids (TSS) achieved a stable value. In this phase, TSS ranged from 0.77 to 3.91 g L^{-1} (Table 2), and no excess sludge was discharged during the entire sludge acclimatization period, except for sludge sampling.

Phase II: MBR start-up and stabilization

In phase II, the sludge was transferred from the inoculation tank to the MBR system, the operation of which spanned 119 days (days 57-175), in the absence of SDZ. During this period, the TSS concentration increased from 1.5 to 18.7 g L^{-1}, but the VSS/TSS ratio kept stable at (0.82 ± 0.07) (Table 3). In this phase, the continuous organic load favored microorganism growth (Metcalf and Eddy, 2003). The MBR was operated without any planned sludge withdrawal except for sludge sampling. Physical and chemical cleanings were carried out during this phase.

Phase III: MBR operation in the presence of SDZ

In this phase, SDZ was added to the reactor at 10 mg L^{-1}, which is about the same magnitude as real antibiotic concentrations found in the wastewater from pharmaceutical formulation facilities. During this period (55 days), sludge was withdrawn frequently in order to maintain TSS in the range 8-12 g L^{-1} and the SRT was 31 days. No chemical or mechanical cleaning was required during this phase.

Table 2. Operating conditions of the pilot-scale MBR system.

Parameter	Phase I (sludge acclimatization)	Phase II (MBR start-up and stabilization)	Phase III (MBR operation in the presence of SDZ)
pH	6.0 ± 0.7	6.3 ± 0.4	6.0 ± 0.5
Temperature (°C)	24.5 ± 2.1	24.4 ± 2.0	22.4 ± 1.7
Q (L h^{-1})	–	10.8 ± 2.6	16.1 ± 2.2
TMP (kPa)	–	13.5 ± 9.9	4.2 ± 3.4
HRT (h)	–	12	12
SRT (days)	–	infinite	31
TSS (g L^{-1})	0.77-3.91	1.51-18.68	5.50-15.64
VSS/TSS	0.88 ± 0.09	0.82 ± 0.07	0.83 ± 0.04
Operation mode of membrane unit	–	run:idle = 8 min:2 min	run:idle = 8 min:2 min

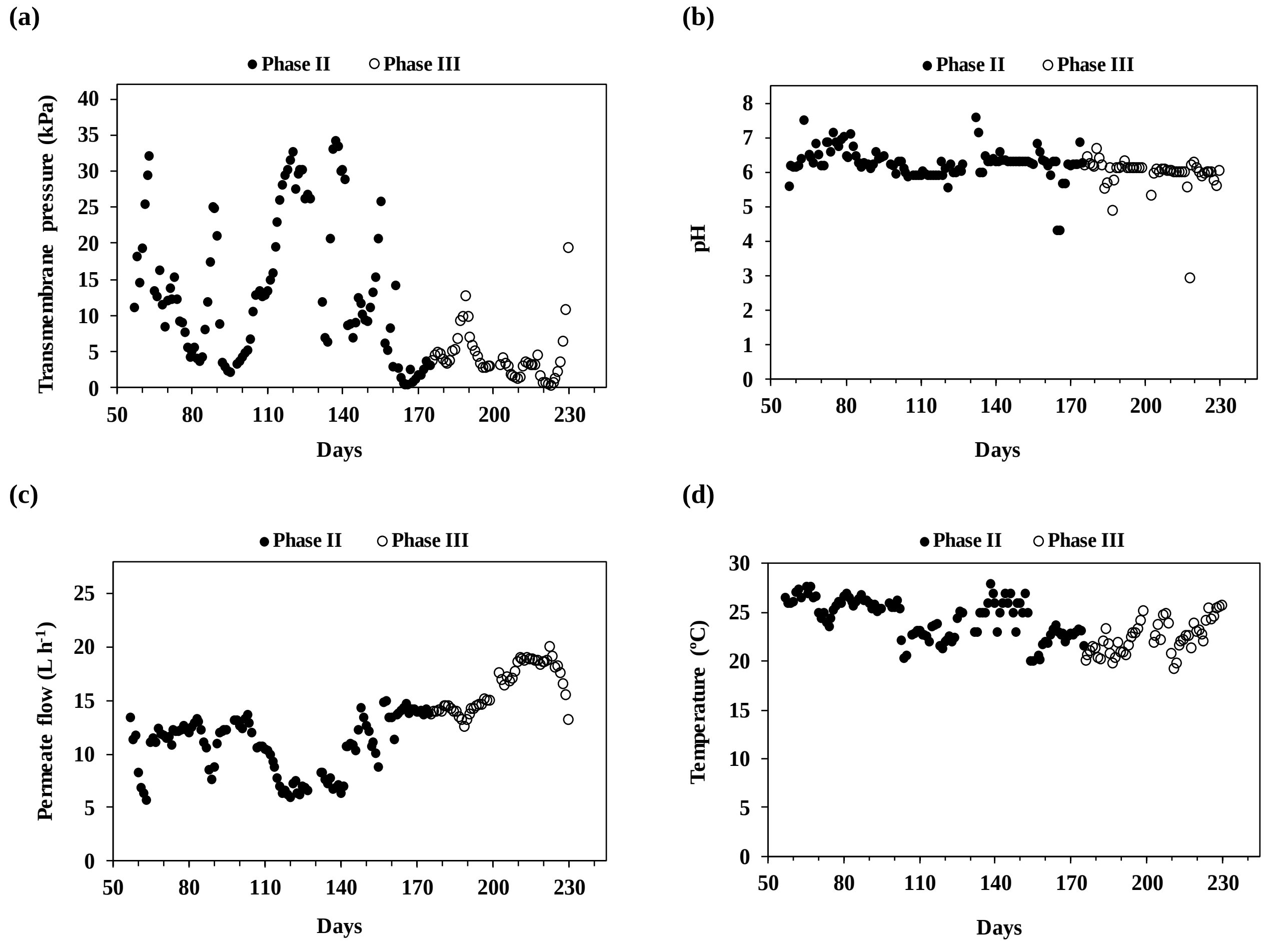

Fig. 1. Time profiles of the transmembrane pressure (TMP) (a), pH (b), permeate flow rate (Q) (c) and temperature (d) during MBR operation (phases II and III). The data points correspond to the daily average of real-time monitored values.

When the operation started, the transmembrane pressure (TMP) varied significantly during phase II (Fig. 1a), possibly due to the adaptation of the sludge to the MBR reactor, while it returned to the ordinary level (< 5 kPa) after physical and chemical cleanings. In this phase, no sludge discards were made leading to an infinite sludge retention time (SRT). In contrast, during phase III, the TMP behaved more stably at about (3.7 ± 2.6) kPa over the first 52 days and then gradually increased towards the end of operation. During phase III, sludge was withdrawn frequently and the SRT was 31 days.

As observed in Fig. 1b, the system pH was maintained in between 6 and 7 throughout the operational period (phases II and III). According to Besha et al. (2017), this is the recommended optimum pH range for microorganisms with regard to total organic carbon (TOC) removal efficiency. However, the MBR removal efficiency is pH-dependent for ionizable organics pollutants, as is the case of SDZ (phase III), which exhibits two pK_a values (2.14 and 6.34, Batista et al. 2014). Tadkaew et al. (2010) studied the effects of mixed liquor with pH in the range 5-9 on the removal of trace organics by a MBR system. The authors reported higher removal efficiencies for sulfamethoxazole, ibuprofen, ketoprofen, and diclofenac (pK_a in the range 4.2-5.8) at pH 5 in comparison to basic conditions, possibly due to the speciation of these compounds. In fact, at pH 5 they exist predominantly as hydrophobic neutral species and, therefore, can sorb onto the activated sludge quite readily, resulting in higher removal efficiencies (Tadkaew et al. 2010).

As shown in Figure 1c, the permeate flow rate varied in the range 5.7-14.9 L h^{-1} during phase II, with an average value of (10.8 ± 2.6) L h^{-1}. In phase III, Q remained in the range of 9.7-20 L h^{-1}, with an average of (16.1 ± 2.2) L h^{-1}. As shown in Figs. 1a and 1c, the variations in TMP were consistent with the variations of the permeate flow.

Finally, Fig. 1d indicates a variation in the range 20-28 °C during MBR operation. It is well known that microorganism growth is affected by temperature; nevertheless, the effects of temperature on the micropollutants removal efficiencies are still not fully understood.

Fig. 2 shows the total (TSS) and volatile suspended solids (VSS) profiles during MBR treatment (phases I, II and III).

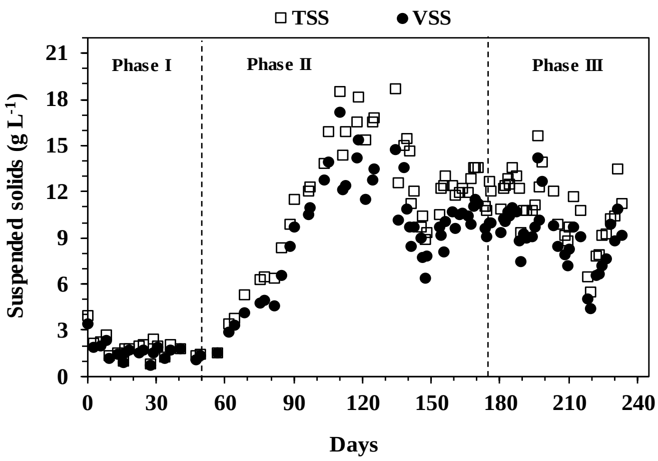

Fig. 2. Time variation of total (TSS) and volatile (VSS) suspended solids during MBR operation (phases I, II and III).

In phase I, TSS and VSS fell within the range (0.77-3.91) g L^{-1} and (0.69-1.55) g L^{-1}, respectively, with an average ratio VSS/TSS = (0.88 ± 0.09) (Table 2 and Fig. 2). In phase II, TSS and VSS concentrations did sharply increase over the first days of operation (days 60-110), with growth of biomass content (TSS) from 1.5 to 18 g L^{-1} in two months. Thereafter, these parameters were controlled by periodic sludge purges to keep TSS values ranging from 8 to 12 g L^{-1}. This range was kept approximately constant in the last phase III. According to Le-Clech et al. (2006), TSS levels between 8 and 12 g L^{-1} do not appear to exhibit a significant effect on membrane fouling. However, high TSS or VSS loadings (12-15 g L^{-1}) is a known cause of significant bio-cake layer development on membranes surface (Scholes et al. 2016).

COD, BOD_5, and TOC were measured for the influent (synthetic wastewater) and permeate during phases II and III (see Table SM_2). In phase II, the mean COD removal efficiency was 93.7% and the permeate COD remained below 197 mg L^{-1}. In phase III, with the addition of SDZ, a very similar mean COD removal efficiency was observed (93.3%). In this phase, the influent COD was (2210 ± 409) mg L^{-1} and the amount of measured permeate COD was (151 ± 36) mg L^{-1}. For BOD_5, the mean removal efficiencies were 91.6% and 94.2% in phases II and III, respectively. The permeate BOD_5 concentrations varied in the ranges 5-85 mg L^{-1} (phase II) and 6-84 mg L^{-1} (phase III); the corresponding average values were (36.0 ± 26.6) and (24.5 ± 29.3) mg L^{-1} for phases II and III, respectively. For TOC, 77.2% and 66.3% was removed during phases II and III, respectively. The average values of permeate TOC were 123 ± 95 and 195 ± 81 mg L^{-1} during phases II and III, respectively. These results are in agreement with Chen et al. (2003), who investigated the performance of a pilot-scale MBR plant equipped with anoxic, aerobic and membrane tanks, with HRT values of 2.89, 9.66, and 0.68 h, respectively. The authors reported COD removals higher than 94%, despite large fluctuations in influent COD (371-2300 mg L^{-1}). For TOC and BOD_5, the authors reported removal efficiencies of 96.3% and 97.6%, respectively.

Phase III of MBR operation consisted in adding SDZ and evaluating its removal. In MBR systems, the main mechanisms responsible for removing pharmaceutical compounds are sludge sorption (adsorption and/or absorption), biodegradation by microorganisms and/or physical retention by membranes (Besha et al. 2017, Tambosi et al. 2010). Fig. 3 shows SDZ concentrations over 55-days of (9.8 ± 0.8) mg L^{-1} (inlet synthetic wastewater), (7.4 ± 1.1) mg L^{-1} (permeate), and (1.1 ± 0.5) mg L^{-1} (sludge). These results indicate that the antibiotic was moderately sorbed onto the sludge (11.3%) and partially degraded by the microorganisms (12.4%), owing to its limited biodegradability, which is affected by SDZ antibacterial activity (Li & Zhang 2010). Thus, approximately 76% of SDZ passed non-degraded through the MBR. In contrast, high TOC (66.3%), BOD5 (94.2%), and COD (93.3%) removals were achieved.

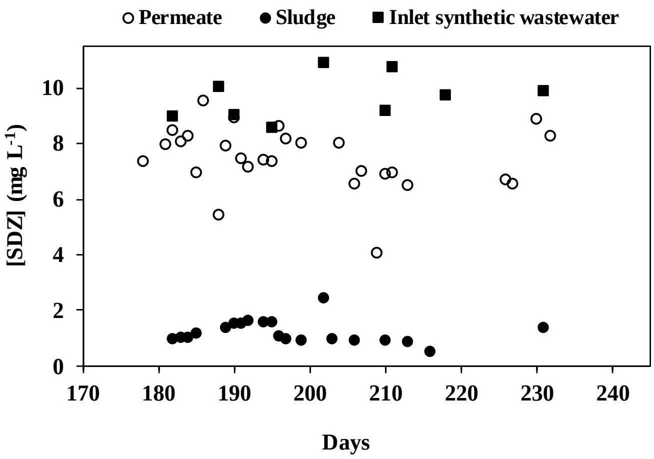

Fig. 3. SDZ removal during MBR treatment.

The physical retention of SDZ molecules by the membranes has not been evaluated in the present work, but it is reasonable to suppose that such a mechanism has not led to SDZ removal. In fact, the molecular weight cut off of ultrafiltration MBR membranes is around 100-200 kDa (Tambosi et al. 2010), while the molar weight of SDZ is 250.3 g mol^{-1} (or 0.250 kDA), such that the antibiotic molecules are capable of crossing the membranes.

Tambosi et al. (2010) studied the removal of pharmaceuticals (acetaminophen, ketoprofen, naproxen, roxithromycin, sulfamethoxazole, and trimethoprim) in two MBR systems with sludge retention times (SRT) of 15 (MBR-15) and 30 (MBR-30) days. The authors found only 55% and 64% sulfamethoxazole (SMX) removal in the MBR-15 and MBR-30 processes, respectively. As remarked by the authors, SMX is hydrophilic (log K_{ow} = 0.89) with two ionizable amine groups (p*K*a = 1.8 and 5.7, Tambosi et al., 2010) and is present predominantly as negatively charged species above pH 5.8. As a consequence, in the MBR systems studied by these authors at pH 7.2, sludge adsorption played a negligible role on the antibiotic removal, as a result of the electrostatic repulsion between both SMX and sludge surface negative charges (Tambosi et al. 2010). These results are in agreement with those we obtained for SDZ, whose pK_a values indicate that for pH > 6.34, sulfadiazine molecules are mostly negatively charged, very hydrophilic and soluble. Our results also agree with those of Xu et al. (2019), who reported that SDZ exhibited relatively low adsorption potential onto activated sludge, with removals below 12.5% after 48 h of contact with the sludge.

The fact that SDZ was not effectively removed in the MBR process suggests the need to employ a polishing step as detailed in the next section.

3.2. Results of polishing technology: MBR+O_3 system

The degradation of SDZ by ozonation as a polishing technology was investigated under different conditions following a Doehlert experimental design (Table 1), using the permeate generated in the MBR system during phase III operation; the response was the pseudo first-order SDZ specific degradation rate (k_{SDZ}, min^{-1}). A good fitting of the experimental data by the response surface model (Equation 1), in terms of the coded variables X_1 (O_3 gas flow rate, L min^{-1}) and X_2 (inlet O_3 concentration, mg L^{-1}) was obtained, with R^2 = 0.994. The analysis of variance (ANOVA) showed that all the effects of the independent variables are statistically significant at 95% confidence level, except for the quadratic terms (X_1^2, X_2^2) (Table SM_3), whose physical meaning also cannot be satisfactorily explained. Therefore, after removing these terms, a new polynomial model (Equation 2) was fitted, for which the determination coefficient decreased slightly (R^2 = 0.952). As indicated by the corresponding ANOVA (Table SM_4) and Pareto chart (Figure SM_2), all model parameters are significant at 95% confidence level.

$$k_{\mathrm{SDZ}}(\mathrm{min}^{-1}) = 1.103 + 0.405X_1 + 0.326X_2 + 0.652X_1X_2 \qquad (2)$$

The response surface corresponding to Equation 1, in terms of the uncoded variables (Figure 4) indicates that the highest SDZ degradation rate was achieved for the combination of the independent variables at their highest values. This corresponds to the highest ozone dose, thus favoring ozone dissolution and increasing its concentration in the liquid. In fact, the ANOVA confirmed the significance of the interaction of both variables, X_1X_2. These results are in accordance with the findings of Garoma et al. (2010), who found that the removal of sulfonamides (sulfadiazine, sulfamethizole, sulfamethoxazole, and sulfathiazole) in aqueous solution was enhanced with the increase of inlet O_3 concentration in the gas bubbled through the liquid, in the range 1.0-3.2 mg L^{-1}. A similar result is reported by Guo et al. (2016) for sulfadiazine degradation in water by the UV/O_3 process ($[SDZ]_0$ =25 mg L^{-1}, UV intensity = 0.3 mW cm^{-2}, pH 7 and O_3 gas flow rate = 0.4 L min^{-1}). The authors related the increased SDZ removal rate with an increase in inlet ozone concentration (30 to 43.8 mg L^{-1}), which promoted ozone-liquid mass transfer.

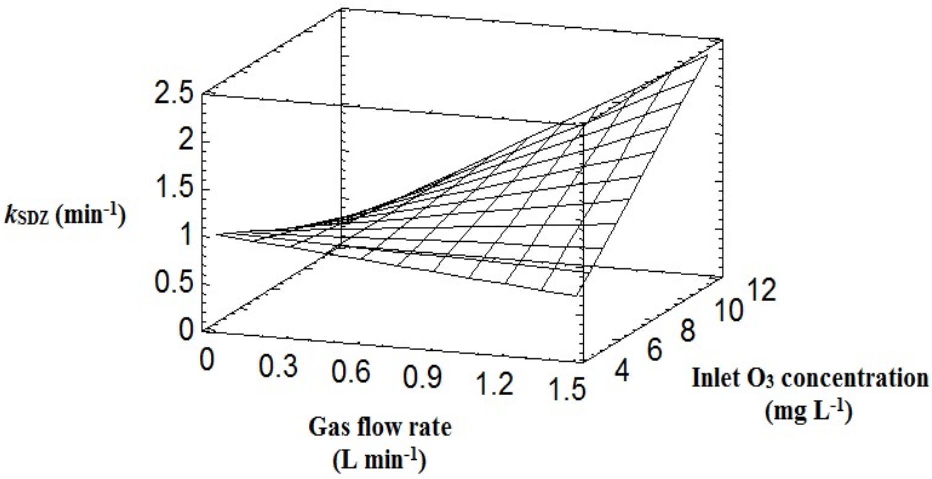

Fig. 4. Response surface obtained for k_{SDZ} (min^{-1}) as a function of gas flow rate (L min^{-1}) and inlet O_3 concentration (mg L^{-1}).

The positive effect of the gas flow rate upon the SDZ removal rate was observed particularly for the highest ozone inlet concentration (Fig. 4). Ji et al. (2018) studied the ozone/zero-valent iron process to treat antibiotic-containing wastewater from a pharmaceutical production facility, and reported a dramatic increase in COD removal with ozone flow rate up to 0.25 L min^{-1}, with no significant changes with a further increase to 0.5 L min^{-1}. According to the authors, this behavior may be related to the limit of mass transfer rate between the gas and liquid phases. Wang et al. (2012) reported a three-fold increase in the pseudo first-order tetracycline degradation rate constants with ozone gas flow rate varying in the range 0.5-0.83 L min^{-1}. The authors associated this behavior with a larger net surface area for mass transfer of the oxidant from the gas to the aqueous phase, therefore increasing the volumetric mass transfer coefficient of ozone. Low mass transfer rates from gas to the liquid phase are a major limitation of ozonation technologies, causing high ozone demands. The selection of the gas-liquid contact device (bubble columns, porous diffusers, packed columns, static mixers, Venturi injectors, among others) can affect the performance of the ozonation system (Gomes et al. 2020), and must therefore be carefully considered.

Fig. 5 shows the values of the SDZ specific degradation rate k_{SDZ} as a function of applied O_3 dose (mg O_3 min^{-1}) for each experimental design condition. The SDZ degradation rate remained practically invariable for lower doses (< 5 mg O_3 min^{-1}), and then increased almost linearly in the range 5-15 mg O_3 min^{-1}. Urbano et al. (2017) evaluated the influence of pH (3-11) and ozone dose (0-46 mg min^{-1}) on sulfaquinoxaline ozonation by a 2^2 experimental design with star points and three replicates of the central point. The results showed that the sulfaquinoxaline removal was enhanced at higher ozone doses.

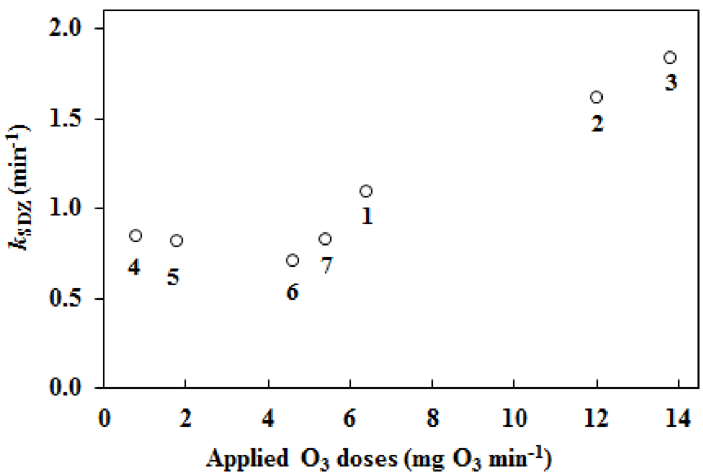

Fig. 5. Variation in the SDZ specific degradation rate as a function of O_3 dose (mg O_3 min^{-1}). Conditions: gas flow rate = 0.1-1.5 L min^{-1}, inlet O_3 concentration = 4-12 mg L^{-1}, $[SDZ]_0$ = (7.68 ± 0.19) mg L^{-1}, pH = 6.28 ± 0.19, 25 °C and 300 rpm. The numbers inside the graph correspond to the experimental runs.

Finally, an additional experiment was performed in duplicate using the synthetic wastewater ($[SDZ]_0$ = (10.6 ± 0.1) mg L^{-1}) fed to the MBR system. The experimental conditions were: O_3 flow rate, 0.8 L min^{-1}; inlet O_3 concentration, 8 mg L^{-1}; pH 5.6; 25 °C; 300 rpm. Figure SM_3 shows that the antibiotic was completely removed after 10 minutes of treatment.

In addition, SDZ, TOC, BOD_5 and DQO removals were monitored during ozonation, MBR, and MBR coupled with ozonation as a polishing configuration, i.e., a single ozonation step following MBR treatment (Table 3).

Table 3. Performance of different processes arrangements: O_3, MBR and MBR+O_3.

	[SDZ] (mg L^{-1})	**TOC (mg L^{-1})**	**BOD_5 (mg L^{-1})**	**COD (mg L^{-1})**	**Turbidity (NTU)**	**BOD_5/COD (%)**
Synthetic wastewater	10.6	672.2	520	1898	3.14	27.4
O_3	0.21	657.0	281	343.9	1.18	81.7
MBR[a]	7.10	24.7	44	130.9	1.71	33.6
MBR+O_3	0	22.9	29	59.6	1.41	48.7

[a] The values correspond to single day measurements.

The results show that ozonation was very effective in removing SDZ from the synthetic wastewater and could lead to virtually complete removal of the antibiotic from contaminated waters. Ozone is well-known for its capacity of mineralizing pharmaceuticals and organic contaminants (Garoma et al. 2010), which can react directly with aqueous O_3 and/or hydroxyl radicals originated from O_3 decomposition. Additionally, in our study, ozonation resulted in a considerable increase in biodegradability of the synthetic wastewater, since the BOD_5/COD ratio increased from 27.4% (synthetic wastewater) to 81.7%. Nevertheless, ozonation resulted in lower removals of TOC (2.3%) and BOD_5 (46%).

Instead, SDZ was partially removed (33%) by the MBR system (HRT = 12 h), probably due to its moderate sorption onto the sludge and limited biodegradability; in contrast, high removals of TOC (96.3%), BOD_5 (91.5%) and COD (93.1%) were achieved. Gobel et al. (2007) also reported the low removal of another sulfonamide antibiotic (sulfamethoxazole) (ca. 37%) through a MBR operating at 13-h HRT.

Finally, the results in Table 3 show that the polishing coupled configuration (MBR+O_3) resulted in the highest overall removal of the parameters monitored: SDZ (100%), TOC (96.5%), BOD5 (94.4%) and COD (96.9%). Furthermore, the ratio BOD_5/COD of the treated permeate increased from 33.6% (MBR) to 48.7% (MBR+O_3); also, the increase in the biodegradability with respect to the untreated synthetic wastewater is remarkable. Ozonation has also shown to be effective as a polishing technology for removing pharmaceuticals from biologically pre-treated wastewater in a single medical section of a hospital (Hansen et al. 2016). In addition, according to Ikehata et al. (2006), the combination (biological+AOP) can avoid the use of exceedingly high oxidant amounts to achieve effective degradation of trace target contaminants.

Conclusions

This study explored the performance of different processes: membranes bioreactor (MBR), ozonation (O_3) and integrated MBR+O_3, for removing the antibiotic sulfadiazine (SDZ) in a synthetic water matrix of industrial interest. The MBR system clearly indicated superior performance compared to ozonation in terms of removals of TOC (96.3%), BOD_5 (91.5%) and COD (93.1%). The antibiotic was moderately sorbed onto the sludge and partially biodegraded. In contrast, SDZ was partially removed by MBR but completely removed in the ozonation step. According to the experimental design used to evaluate the ozone-driven degradation of the remaining SDZ in the MBR permeate, the influence of the ozone gas flow rate upon SDZ removal was more important than that exhibited by the inlet gaseous ozone concentration, with pseudo first-order SDZ specific degradation rates increasing linearly for applied ozone doses higher than 5 mg min^{-1}. Likewise, given the importance of ozone mass transfer rates from gas to the liquid phase in ozonation technologies, the selection of gas-liquid contactors should be further investigated.

Finally, the MBR+O_3 process revealed to be an effective method to degrade SDZ from synthetic wastewater in comparison with MBR and ozonation alone, showing excellent performances for all the parameters monitored: SDZ (100%), TOC (97%), BOD_5 (94.4%), COD (97%), and DBO_5/COD (48.7%). Therefore, the results confirm the synergistic effect between the biological treatment and advanced chemical oxidation.

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SUPPLEMENTARY MATERIAL

Table SM_1. Composition of the synthetic wastewater for MBR operation.

Compound	Formula	Concentration (mg L^{-1})
	Macro-components	
Sodium chloride	NaCl	1000
Citric acid	$C_6H_8O_7$	50
Ascorbic acid	$C_6H_8O_6$	30
Sucrose	$C_{12}H_{22}O_{11}$	1200
Sodium phosphate dibasic	Na_2HPO_4	44
Urea	$(NH_2)_2CO$	51
	Micro-nutrients	
Manganese sulfate monohydrate	$MnSO_4.H_2O$	10
Ammonium heptamolybdate tetrahydrate	$(NH_4)_6Mo_7O_{24}.4H_2O$	1.5
Copper (II) sulphate	$CuSO_4$	2
Zinc sulfate heptahydrate	$ZnSO_4.7H_2O$	7.5

Table SM_2. Performance of the MBR pilot system.

Parameter	Phase II			Phase III		
	Synthetic wastewater (mg L^{-1})	Permeate (mg L^{-1})	Removal (%)	Synthetic wastewater (mg L^{-1})	Permeate (mg L^{-1})	Removal (%)
COD	1739.6 ± 567.4	109.0 ± 20.7	93.5	2210.5 ± 408.6	151.2 ± 35.8	93.3
BOD_5	489.5 ± 65.4	36.0 ± 26.6	91.6	452.8 ± 51.3	24.5 ± 29.3	94.2
TOC	542.8 ± 53.2	123.8 ± 94.8	77.2	580.5 ± 58.5	195.4 ± 80.8	66.3

Table SM_3. Analysis of variance (ANOVA) for the pseudo first-order SDZ specific degradation rate (k_{SDZ}, min^{-1}) obtained for the ozone-driven SDZ degradation. Conditions: $[SDZ]_0$ = (7.68 ± 0.19) mg L^{-1}; O_3 gas flow rate = 0.1-1.5 L min^{-1}; inlet O_3 concentration = 4-12 mg L^{-1}; pH = (6.28 ± 0.19); 25 °C.

Source	Sum of Squares	Degrees of Freedom	Mean Square	*F*-Ratio	*p*-Value
X_1: O_3 **gas flow rate**	0.492	1	0.492	203.15	0.0007
X_2: **inlet O_3 concentration**	0.319	1	0.319	131.79	0.0014
X_1^2	0.023	1	0.023	9.48	0.0542
X_2^2	0.017	1	0.017	6.94	0.0781
$X_1 X_2$	0.319	1	0.319	131.79	0.0014
Total Error	0.007	3	0.002		
Total (corr.)	1.187	8			
R^2	0.994				

Table SM_4. Analysis of variance (ANOVA) for the pseudo first-order SDZ specific degradation rate (k_{SDZ}, min^{-1}) obtained for the ozone-driven SDZ degradation, without the effects of X_1^2 and X_2^2. Conditions: $[SDZ]_0$ = (7.68 ± 0.19) mg L^{-1}; O_3 gas flow rate = 0.1-1.5 L min^{-1}; inlet O_3 concentration = 4-12 mg L^{-1}; pH = (6.28 ± 0.19); 25 °C.

Source	Sum of Squares	Degrees of Freedom	Mean Square	*F*-Ratio	*p*-Value
X_1: O_3 **gas flow rate**	0.492	1	0.492	43.26	0.0012
X_2: **inlet** O_3 **concentration**	0.319	1	0.319	28.06	0.0032
X_1 X_2	0.319	1	0.319	28.06	0.0032
Total Error	0.057	5	0.011		
Total (corr.)	1.187	8			
R^2	0.952				

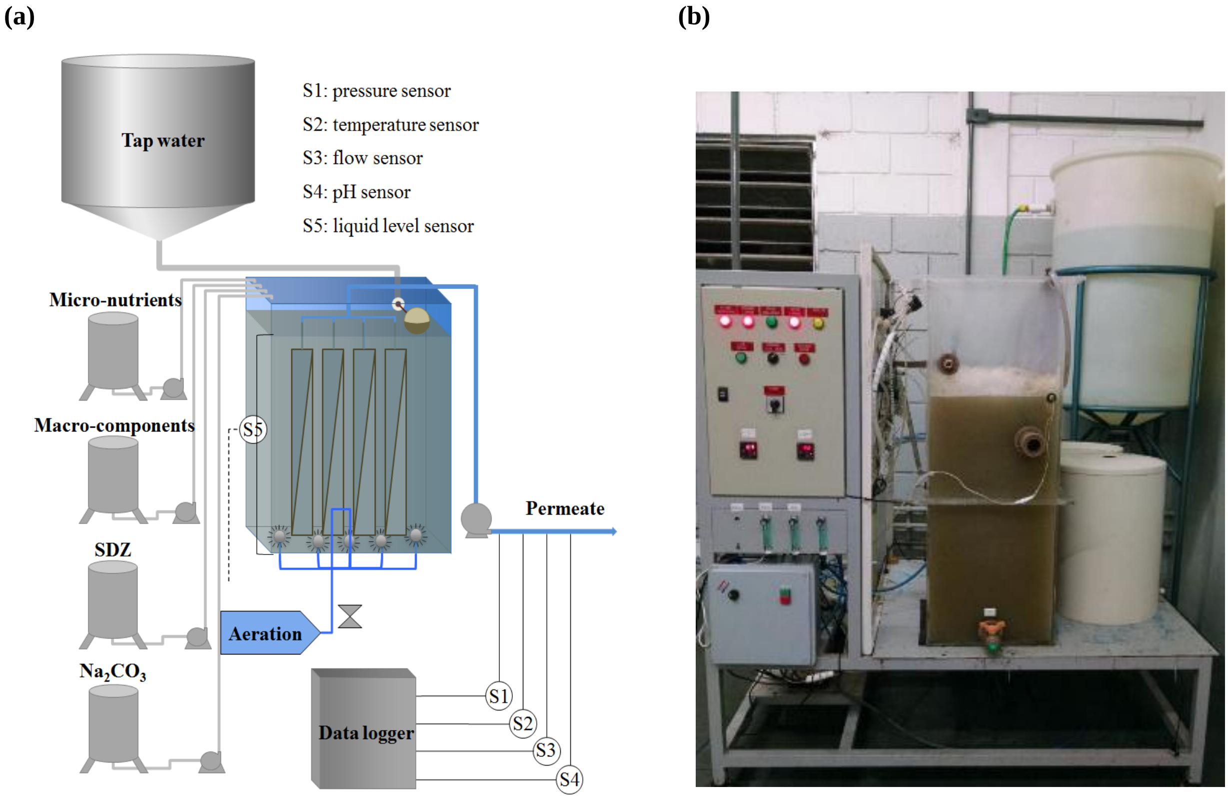

Figure SM_1. Simplified scheme (a) and photograph (b) of the MBR system used in this study.

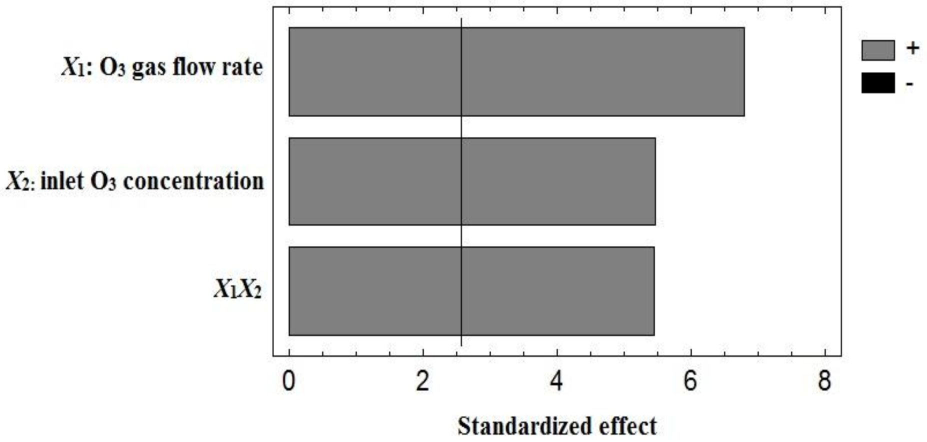

Figure SM_2. Pareto chart for the pseudo first-order SDZ specific degradation rate (k_{SDZ}, min^{-1}) obtained for the ozone-driven SDZ degradation, without the effects of X_1^2 and X_2^2. Conditions: $[SDZ]_0$ = (7.68 ± 0.19) mg L^{-1}; O_3 gas flow rate = 0.1-1.5 L min^{-1}; inlet O_3 concentration = 4-12 mg L^{-1}; pH = (6.28 ± 0.19); 25 °C.

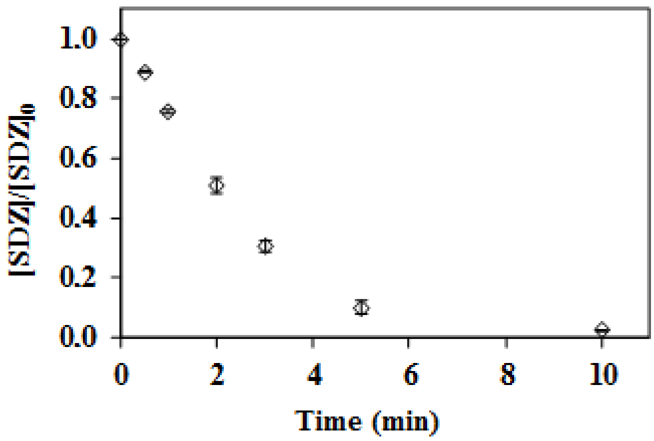

Fig. SM_3. Removal of SDZ from the synthetic wastewater by the ozonation process. Conditions: O_3 gas flow rate, 0.8 L min^{-1}; inlet O_3 concentration, 8 mg L^{-1}; $[SDZ]_0$ = (10.6 ± 0.1) mg L^{-1}; pH 5.6; 25 °C; 300 rpm.