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Treating wastewater from a pharmaceutical formulation facility by biological process and ozone

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ARTICLE INFO

Article history:
Received 19 December 2012
Received in revised form
20 March 2013
Accepted 27 April 2013
Available online 16 May 2013

Keywords:
Pharmaceutical
Formulation facility
Ozone
Carbamazepine
Venlafaxine

ABSTRACT

Wastewater from a pharmaceutical formulation facility (TevaKS, Israel) was treated with a biological activated-sludge system followed by ozonation. The goal was to reduce the concentrations of the drugs carbamazepine (CBZ) and venlafaxine (VLX) before discharging the wastewater to the municipal wastewater treatment plant (WWTP). Both drugs were detected at extremely high concentrations in TevaKS raw wastewater ([VLX] = 11.72 ± 2.2 mg/L, [CBZ] = 0.84 ± 0.19 mg/L), and resisted the biological treatment. Ozone efficiently degraded CBZ: at an O_3 dose-to-dissolved organic carbon ratio of 0.55 (O_3/DOC), the concentration of CBZ was reduced by >99%. A lower removal rate was observed for VLX, which was decreased by ~98% at the higher O_3/DOC ratio of 0.87. Decreasing the pH of the biologically treated effluent from 7 to 5 significantly increased the ozone degradation rate of CBZ, while decreasing the degradation rate of VLX. Ozone treatment did not alter the concentration of the effluent's DOC and filtered chemical oxygen demand (COD_f). However, a significant increase was recorded (following ozonation) in the effluent's biological oxygen demand (BOD_5) and the BOD_5/COD_f ratio. This implies an increase in the effluent's biodegradability, which is highly desirable if ozonation is followed by a domestic biological treatment. Different organic byproducts were formed following ozone reaction with the target pharmaceuticals and with the effluent organic matter; however, these byproducts are expected to be removed during biological treatment in the municipal WWTP.

1. Introduction

Pharmaceutical manufacturing facilities (which mainly include production and formulation facilities) are typically considered insignificant sources of pharmaceutical pollution in the environment (Fick et al., 2009). The main arguments supporting this hypothesis are the well-controlled production processes, the strict regulations (mainly in the USA) and the high cost of the active ingredients (facilitating their recovery), all of which would theoretically guarantee a negligible discharge of active substances into the wastewater (as cited by Larsson et al., 2007). Surprisingly, this hypothesis has only recently been challenged.

A study conducted in the Patancheru industrial area of India, a major worldwide production site of generic drugs, detected extremely high concentrations of various pharmaceutical residues in the local secondary effluents. These residues originated from wastewaters of native drug manufacturers (Larsson et al., 2007). The most abundant drugs were detected at levels higher than 100 μg/L—well above the highest concentration previously reported for any pharmaceutical in any wastewater effluent (e.g. Andreozzi et al.,

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http://dx.doi.org/10.1016/j.watres.2013.04.059

2003). Markedly high concentrations were found for different fluoroquinolone antibiotics. For example, ciprofloxacin was detected at concentrations as high as 31 mg/L. In terms of drug load, the release of ciprofloxacin through the wastewater treatment plant (WWTP) effluent corresponded to approximately 45 kg of active ingredient per day, which is equivalent, for example, to the total consumption of ciprofloxacin in Sweden over a period of 5 days (as cited by Larsson et al., 2007). Furthermore, the detected concentrations of ciprofloxacin were several orders of magnitude higher than the drug's toxicity values for *Microcystis aeruginosa* (0.017 mg/L) and *Lemna minor* (0.203 mg/L), two widespread aquatic organisms, commonly used in eco-toxicity tests (Robinson et al., 2005). The effluent's indirect discharge into nearby rivers resulted in drug pollution of various local water sources (including groundwater wells) (Fick et al., 2009). Moreover, outcomes of a previous study (Greenpeace, 2004) demonstrated that 40 years of discharging drugs and other industrial waste into native environments has resulted in severe environmental and human health hazards in that region.

Pharmaceutical pollution originating from manufacturing facilities is not limited to India, where regulations are not strictly enforced, but is also found in Europe (Hoerger et al., 2009) and the USA (Phillips et al., 2010). A survey conducted in the USA during 2004–2009 analyzed effluent from municipal WWTPs, receiving significant and fixed wastewater flows (>20% of plant flow) from pharmaceutical formulation facilities. The effluents contained drugs at concentrations up to 1000 times higher than effluent from a WWTP treating regular domestic and hospital wastewater (Phillips et al., 2010). Thus, manufacturing facilities can cause local drug pollution above and beyond the pollution caused by household discharge. It has been suggested that these wastewaters be treated on site (at the manufacturing plant) to reduce the environmental load of drug residues.

Biologically based treatments, recommended by the EPA as the best available technology for treating pharmaceutical wastewater (EPA, 1998), are often inefficient at removing a large variety of compounds (e.g. Clara et al., 2004). Other non-biological treatments, such as ozone and advanced oxidation processes (AOPs), have been shown to efficiently remove pharmaceuticals from domestic wastewater effluent (after biological treatment) (Dodd et al., 2006; Wert et al., 2009; Zimmermann et al., 2011). For example, Hollender et al. (2009) has investigated the elimination of approximately 55 micropollutants by ozone, with diverse functional groups, from secondary municipal wastewater effluent. They found that compounds with activated aromatic moieties, amine functions, or double bonds as sulfamethoxazole and carbamazepine, with known high second-order reaction rate constants with ozone ($>10^4$ 1/M s), were eliminated to concentrations below the detection limit for an O_3/dissolved organic carbon (DOC) ratio of only 0.47. Many other compounds, such as the antidepressant venlafaxine (amines), were efficiently eliminated at a slightly higher O_3/DOC ratio of 0.62.

Clearly, ozone and AOPs can be used efficiently to treat domestic wastewater effluent; however, only a few studies have evaluated their potential to treat wastewater directly from the pharmaceutical industry. Arslan Alaton et al. (2004) found ozone and ozone/H_2O_2 to be efficient for treating penicillin formulation wastewater, with COD removal of up to 83%, and a significant increase in the wastewater biodegradability (calculated as the COD/BOD_5 ratio).

The objective of the present study was to demonstrate the feasibility of a biological treatment followed by an optimized ozonation process to treat wastewater from the pharmaceutical manufacturing facility Teva Kefar-Saba (TevaKS) in Israel. Teva Pharmaceuticals Industries Ltd. is one of the largest pharmaceutical companies in the world, with over 50 production plants worldwide. TevaKS is a formulation facility where bulk pharmaceutically active ingredients are converted into a finished dosage form of the drug for consumption (e.g. capsules or tablets).

The specific goal of this study was to determine the efficiency of the proposed combined treatment in removing carbamazepine (CBZ; antiepileptic) and venlafaxine (VLX; antidepressant) from TevaKS wastewater, prior to its discharge to the local municipal WWTP. The target drugs were selected due to their high production volumes in the plant and their low removal by municipal WWTPs (e.g. Clara et al., 2004; Gasser et al., 2012).

2. Materials and methods

2.1. Standards and reagents

Carbamazepine (CBZ) standard (>99% purity) was obtained from Sigma–Aldrich, and VLX (>99% purity) from Teva Pharmaceuticals Ltd. Para-chlorobenzoic acid (pCBA) was used to determine $HO^{\bullet}$ radical exposure (Sigma–Aldrich, Germany). LC-grade acetonitrile, methanol and water were purchased from Bio-Lab (Jerusalem, Israel). All chemicals were used as obtained and working solutions were prepared with deionized (DI) water (Direct-Q3 UV system, Millipore).

2.2. Wastewater samples

TevaKS has a separate wastewater system for the manufacturing process and for sanitation (e.g. toilets). Wastewater from all manufacturing lines is collected in an equalization tank (also used as an oil separator), passes through a sedimentation basin to remove solids, and is finally discharged to the municipal WWTP at a flow rate of 200–400 m^3/day. Grab samples were taken from the equalization tank during the months of March and May 2012, transported refrigerated to the laboratory and stored at 4 °C until used.

2.3. Biological treatment

The biological treatment was carried out in an aerobic bench-scale activated-sludge continuous stirred-tank reactor (CSTR) with solids recycling, aiming at removing carbonaceous BOD (and not nitrification). Prior to the treatment, solids were removed from the wastewater by sedimentation and the pH was adjusted to ~7. Phosphorous (as H_3PO_4) and ammonia (as NH_4Cl) were added to the wastewater to achieve residual concentrations (in the treated effluent) of at least 3 and 15 mg/L, respectively. The CSTR reactor consisted of a 10-L aeration tank

and a 4.3-L sedimentation tank. The reactor was continuously aerated through a diffuser located at the bottom of the aeration tank, to keep dissolved oxygen level higher than 3 mg/L. The temperature in the reactor was kept constant at 23 ± 1 °C. A peristaltic pump was used for continuous wastewater feeding at a flow of 1 L/day, providing a hydraulic retention time of approximately 10 days. Solids retention time (SRT) and mixed liquor suspended solid (MLSS) concentration were approximately 30 d and 1000 mg/L respectively. Operation of the reactor was initiated by the addition of approximately 1 L of recirculated sludge from a full-scale municipal WWTP and the feeding of wastewater, followed by a sludge-acclimatization period of 2 weeks.

2.4. Ozone treatment

Ozone experiments were performed in a thermostat-controlled (22 °C) 1-L glass cylindrical semi-continuous batch reactor (Ace-glass, NJ). Prior to the ozone experiments, the effluent was filtered through glass-fiber filters with an average pore size of 1.2 μm to remove particulate matter, and the pH was adjusted to either 7 or 5 (using HCl or NaOH). The pH of the effluent remained constant throughout ozonation. Ozone experiments were conducted by adding the effluent to the reactor and sparging it with an ozonized oxygen stream (flow rate 1 L/min, ozone gas concentration ~20 mg/L), using a diffuser located at the bottom of the reactor. Another tube carried off gases from the headspace of the reactor to the ozone destructor. The tested solution was constantly mixed via magnetic stirring. For most analysis, 5 mL samples were taken periodically during ozonation. For specific analysis which requires a larger volume (i.e. solid-phase extraction and BOD), the entire volume of the effluent was taken after applying a known ozone dose.

Competition kinetics was used to determine the second-order rate constant for the reaction of VLX with ozone – $k_{O3,VLx}$ (1/M s), using carbamazepine (CBZ) as a reference compound (Huber et al., 2003). In this experiment, ozone stock solution (~20 mg/L) was first prepared in cold DI water. Different volumes of the stock solution were added to five separate beakers, containing phosphate buffer (5 mM, pH 7), t-butanol (10 mM) and equal concentrations of VLX and CBZ (~4.5 μM each), to achieve ozone doses of 0.6–3.8 μM. The beakers were continuously mixed until all ozone was consumed, and the remaining concentrations of both compounds were analyzed by HPLC/MS.

2.5. Analytical methods

2.5.1. Ozone measurements

Ozone concentration in the gas influent and effluent of the reactor were measured by an ozone gas analyzer (BMT 963, Germany). Ozone in the aqueous solution was measured with a dissolved O_3 sensor (WADIS310, Walchem Corporation, MA) and the indigo method.

2.5.2. Sample extraction

To detect the pharmaceuticals present at low concentrations, samples were pre-concentrated by solid-phase extraction (SPE). SPE was performed using 500 mg/6 mL Oasis-HLB cartridges connected to a 24-port SPE manifold and vacuum pump. The SPE cartridges were conditioned with 4 mL heptane, 4 mL acetone and 10 mL MeOH. Extraction of pre-filtered (0.45 μm) 0.3-L effluent samples was carried out at a flow rate of approximately 10 mL/min. After loading the samples, the cartridges were air-dried for 20 min. The analytes were eluted with 20 mL MeOH, collected in 20-mL glass tubes and dried to zero under 5/9 nitrogen stream. Finally, the extracts were reconstituted with 1 mL HPLC-grade water (with 10% MeOH) and transferred to HPLC vials.

2.5.3. Sample analysis

CBZ and VLX were detected and quantified by HPLC (Agilent 1100) and MS (Finnigan LCQ) using an ACE-RP C18 250 × 2.1 mm column. The column temperature was 38 °C, the flow rate was 0.5 mL/min and the injected volume was 100 μL. The HPLC mobile phase consisted of water (A) and methanol (B), adjusted to pH 3 by the addition of formic acid. The mobile phase eluent gradient started with 40% eluent B, followed by a 15-min linear gradient to 90% B, a 4-min isocratic elution and a 2-min linear gradient back to 40% B, maintained for 4 min for equilibration. The flow from the HPLC was passed through a split connector with 60 μL/min of effluent introduced into the MS interface. The mass spectrometer was used in MS/MS, positive ESI, multiple reaction monitoring (MRM) mode, and the recorded fragment ions were m/z^+ 194.5 for CBZ and m/z^+ 260.2 for VLX. HPLC conditions for pCBA are described in details elsewhere (Lester et al., 2010).

Volatile organic oxidation byproducts were detected by GC–MS (Agilent model 7890/5975c, column ZB-624, 60 m, 0.25 mm I.D., 3 μm film thickness), using headspace technique for sample introduction (LOD 35 μg/L). These analyses were conducted in accordance with the EPA methodology (method 8260), used to determine volatile organic compounds in a variety of waste matrices.

2.5.4. Analysis of wastewater-quality parameters

Total organic carbon (TOC) in the wastewater was measured using a TOC analyzer (Torch, TeledyneTekmar, OH). To measure dissolved organic carbon (DOC), samples were first filtered at 0.45 μm (APHA, method 5310B). The UV absorbance at 254 nm was measured using UV–Vis spectrophotometer (Varian, Cary 100BIO, Victoria, Australia). BOD analysis (with seeding) was conducted according to standard method SM5210B. Other wastewater parameters were analyzed using standard methods (APHA, 2005).

3. Results and discussion

3.1. Detection of the drugs in raw wastewater

CBZ and VLX were detected in TevaKS raw wastewater at concentrations of 0.84 ± 0.19 and 11.72 ± 2.2 mg/L, respectively (Table 1). Taking into account an average wastewater flow rate of 300 m^3/day, the detected concentrations correspond to discharge loads of approximately 0.25 (CBZ) and 3.5 (VLX) kg active pharmaceutical ingredient per day. For comparison, VLX was detected in raw wastewater and treated effluent of the Shafdan WWTP (which is not the WWTP

Table 1 – Efficiency of the biological treatment.

Parameter	Wastewater	Effluent	% Removal
TOC (mg/L)	1698 (±308)	224 (±68)	87
COD (mg/L)	4765 (±1405)	741 (±253)	84
BOD_5 (mg/L)	634 (±100)	48.4 (±20)	92
BOD_5/COD	0.13	0.06	
NH_4^+–N	23.5 (±8)	17.6 (±13)	25
pH	10.2 (±0.9)		
CBZ (mg/L)	0.84 (±0.19)	0.83 (±0.06)	<5
VLX (mg/L)	11.72 (±2.2)	11.34 (±1.1)	<5

treating TevaKS) at a concentration of ~0.2 μg/L (Gasser et al., 2012), equivalent to a daily discharge of approximately 0.08 kg (about 40-fold lower than TevaKS). The Shafdan is the largest WWTP in Israel, treating wastewater from over two million people in the Tel Aviv metropolis. This comparison exemplifies the huge contribution of TevaKS to environmental drug pollution, compare to a large municipal WWTP. Obviously, the suggested on-site treatment to remove VLX and CBZ from TevaKS wastewater holds high potential for reducing the existing environmental load for these drugs.

3.2. *Biological treatment*

The main sources of wastewater in formulation facilities are floor and equipment wash water and spills. This wastewater is typically characterized by low BOD_5 and COD (average $BOD_5 < 600$ mg/L; COD < 900 mg/L) and high biodegradability relative to other drug-production activities such as chemical synthesis (Browner et al., 1998). Table 1 presents the concentrations of the main parameters in TevaKS wastewater, before (wastewater) and after (effluent) being subjected to the bench-scale activated-sludge CSTR system, demonstrating the efficiency of the biological treatment.

TevaKS raw wastewater contains exceptionally high COD levels and low BOD_5/COD ratio (Table 1). According to the TevaKS technical staff, the reason for the high organic load is the low volume of water used for equipment washing ("water-saving" policy).

Despite the relatively low BOD_5/COD ratio of the raw wastewater, high removal of COD and TOC was achieved in the biological treatment (Table 1). This indicates that the wastewater's COD is mainly composed of biodegradable compounds (e.g. organic solvents). In addition, concentrations of CBZ and VLX remained almost constant during the treatment with less than 5% removal. Both CBZ and VLX are known for their low biodegradability and low removal in WWTPs (Kasprzyk-Hordern et al., 2010; Keen et al., 2012).

3.3. *Ozone treatment*

3.3.1. *Removal of the pharmaceuticals*

The main wastewater parameters affecting ozone treatment are dissolved organic matter (DOM) and nitrite (NO_2^-) (Wert et al., 2009). The concentration of DOM in the effluent was ~175 mg DOC/L, while NO_2^- concentration was negligible (<0.5 mg/L). Removal of the pharmaceuticals during ozonation is therefore presented as a function of the ratio of O_3 dose to DOC (O_3/DOC) (Fig. 1). O_3 dose was calculated as the difference between ozone concentrations in the gas influent and effluent from the reactor.

CBZ was quickly degraded by ozone: at an O_3/DOC ratio of 0.55, its concentration was reduced by >99% (from 0.83 to 0.001 mg/L). Further application of ozone resulted in removal of CBZ to below the method's limit of detection (<0.05 μg/L). A slightly lower removal rate was observed for VLX which was decreased by approximately 98% (from 11.72 to 0.20 mg/L) at an O_3/DOC ratio of 0.87.

Wert et al. (2009) found more than 95% removal of CBZ during ozonation of different tertiary domestic wastewater effluents at an O_3/TOC ratio of 0.6. Others have shown complete CBZ removal (below detection limits) and 99% removal of VLX from domestic effluent at an O_3/DOC ratio of 0.47 and ~0.6, respectively (Hollender et al., 2009). The comparable O_3/DOC ratios required to remove the tested pharmaceuticals from TevaKS wastewater and different domestic effluents implies high similarity in the DOM's reactivity toward ozone between TevaKS and domestic wastewaters.

Ozone degradation of organic pollutants depends strongly on the pollutants' reaction rate with molecular ozone. A direct correlation usually exists between the removal of a compound during wastewater ozonation and the compound's second-order reaction rate constant with ozone $k_{O3,pollutant}$ (1/M s), especially for ozone-reactive pollutants (Zimmermann et al., 2011). The rate constant for the reaction of CBZ with ozone has been reported as 3×10^5 1/M s (Huber et al., 2003). For VLX, the rate constant ($k_{O3,VLx}$) was calculated herein to be 3.3×10^4 1/M s, using competition kinetics, with CBZ as a reference compound. The higher rate constant of CBZ (over VLX) might explain its enhanced removal during the treatment.

3.3.2. *Influence of pH on pharmaceutical removal*

pH of the treated water can potentially influence ozone degradation of organic pollutants by affecting both ozone decomposition and the reaction rate of ozone with the target pollutant. Decreasing the pH of TevaKS effluent from pH 7 to 5 significantly enhanced the degradation of CBZ, whereas the degradation rate of VLX was slightly decreased (Fig. 2).

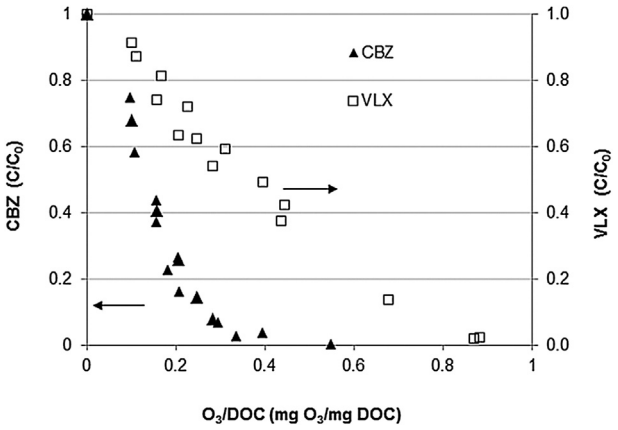

Fig. 1 – Removal of VLX and CBZ from TevaKS effluent (pH 7) as a function of O_3/DOC ratio. Data taken from two separated experiments.

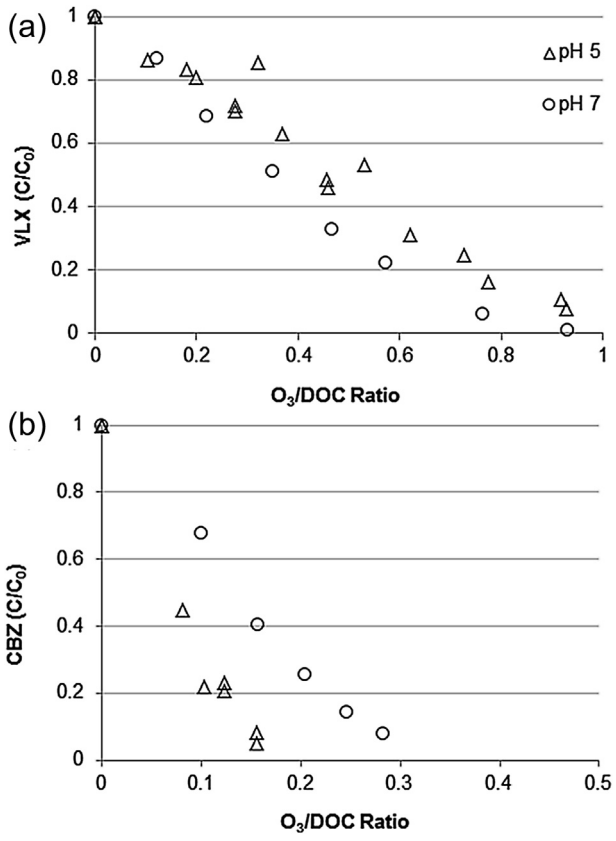

Fig. 2 – Degradation of (a) VLX and (b) CBZ in the effluent as a function of O_3/DOC ratio at pH 5 and 7. Data for pH 5 is taken from two separated experiments. Data for pH 7 are presented using representative results from Fig. 1.

The decomposition of ozone in water is initiated by the reaction of O_3 with hydroxide ions (OH^-) and/or with reactive moieties in the DOM. The OH^--initiated path is highly pH-dependent, with higher stability of O_3 at lower pH (Staehelin and Hoigne, 1982). Direct reaction of ozone with DOM may also contribute to the increase in ozone stability at low pH, since lower reactivity of ozone is expected with protonated acid and phenol moieties in the DOM (than with the deprotonated forms) (Elovitz et al., 2000). Therefore, the increase in O_3 stability probably causes the enhanced removal of CBZ at pH 5. It should be noted, however, that dissolved ozone could not be detected at either pH.

VLX is a tertiary amine with pKa of 9.3 (Gasser et al., 2012). Reaction of non-protonated amine with ozone is much faster than that of the protonated form (Huber et al., 2003); therefore, the reaction rate of VLX with ozone is expected to increase with increasing solution pH, resulting in faster degradation of the drug.

3.3.3. *HO• radical exposure*

Organic contaminants are degraded during ozone application through two main pathways: direct oxidation by molecular ozone and indirect HO• radical (hydroxyl radical) oxidation. The contribution of HO• to the removal of VLX and CBZ was assessed by adding the probe compound pCBA to the effluent before ozonation ($C_0 = 0.5$ mg/L), and following its disappearance. Degradation of pCBA during ozone treatment occurs almost exclusively through its reaction with HO• ($k_{pCBA,O3} < 0.15$ 1/M s; Yao and Haag, 1991), and therefore its removal provides a tool to measure HO• exposure.

Concentration of pCBA remained constant throughout the ozonation process up to 1 mg O_3/mg DOC (results not shown), indicating that HO• exposure was negligible, and that degradation of the pharmaceuticals occurred primarily through their direct reaction with molecular ozone. In a different experiment (without pCBA), 0.2 M t-butanol was added to the effluent before ozonation to scavenge HO• radicals. Degradation rate of CBZ and VLX was not affected by the addition of t-butanol, confirming the negligible contribution of HO• to the drugs' removal.

The insignificant HO• exposure measured herein implies that concentrations of other pollutants, which resist direct ozone oxidation, are unlikely to be reduced during ozonation of TevaKS effluent. These results differ from what is typically observed during municipal effluent ozonation, where ozone-resistant pollutants (e.g. X-ray contrast media) can be removed as much as 50% at an ozone dose of ~1 mg O_3/mg DOC (e.g. Hollender et al., 2009; Wert et al., 2009). The reasons for the low HO• exposure in TevaKS effluent are probably low HO• formation yield and/or high HO• scavenging by effluent constituents (e.g. DOM). For example, acetone, which is a known HO• scavenger ($k_{acetone,•OH} = 1 \times 10^8$ 1/M s; Buxton et al., 1988), was detected in the effluent at the relatively high concentrations of >7 ppm.

3.3.4. *Effect of ozone on the effluent's physicochemical parameters*

Ozone's effect on various effluent parameters was examined. Concentrations of DOC, COD_f (filtered at 0.45 μm) and ammonia were constant throughout the effluent's ozonation (up to 1 mg O_3/mg DOC). Oxidation of ammonia by ozone is a slow process with a second-order rate constant of 20 1/M s (von Gunten, 2003). The decrease in DOC concentration during ozonation is usually insignificant at typical O_3 doses for wastewater treatment (e.g. Bahr et al., 2007). Changes in COD (and COD_f) concentrations by ozone vary with wastewater type and origin. For example, different studies have shown a wide range of COD-removal rates, from 8% to 88%, during ozone treatment of domestic wastewater effluent (Paraskeva et al., 1998; Martinez et al., 2001), while others have shown negligible degradation of COD by ozone when treating effluent from industrial production of the antibacterial drug nalidixic acid (Laera et al., 2012).

Ozone oxidation can potentially increase the biodegradability of organic matter and, as a result, increase the BOD_5 of the effluent. Indeed, applying ozone up to 1 mg O_3/mg DOC increased the BOD_5 of the treated effluent from 79 to 251 mg/L (Fig. 3). Similar trends have been observed for both domestic (e.g. Schumacher et al., 2004) and industrial (e.g. Arslan Alaton et al., 2004) wastewater effluent. Increasing the biodegradability of the effluent is of particular interest when ozonation is followed by a biological treatment, as in the case of TevaKS, where the effluent is further discharged to a municipal WWTP.

The decrease in the drugs' concentrations during ozonation, the stable concentrations of DOC and COD_f, and the

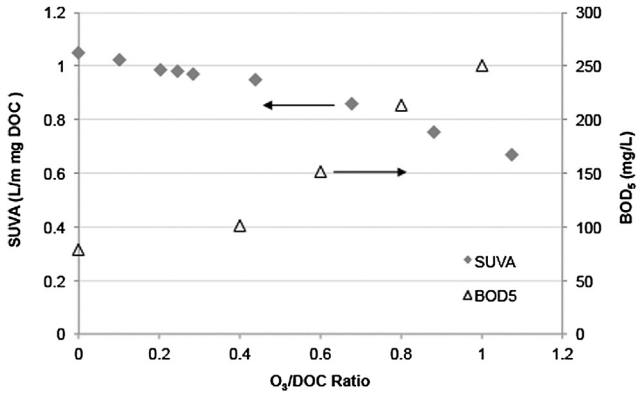

Fig. 3 – SUVA and BOD_5 concentrations *vs.* O_3/DOC ratio during ozonation of TevaKS effluent.

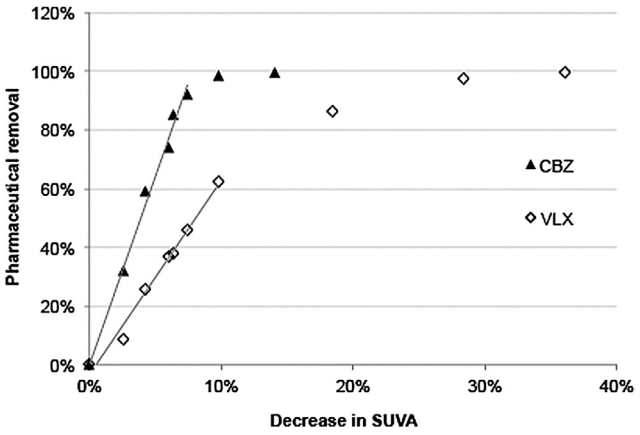

Fig. 4 – Removal of VLX and CBZ *vs.* the decrease in SUVA during TevaKS ozonation.

increase in BOD_5, suggest that the target pharmaceuticals are not fully mineralized by ozone, but rather oxidized to biodegradable organic intermediates.

In addition, ozone sharply reduced the specific UV absorbance (SUVA) of the effluent at 254 nm (the ratio of UV absorbance to DOC; L/m mg DOC) (Fig. 3). A decrease in SUVA during ozonation is a well-documented phenomenon, indicating a decrease in the aromatic carbon content of the effluent due to ozone attack on conjugated systems (e.g. Wert et al., 2009). The decrease in UV absorbance (at 254 nm) of the effluent has been previously proposed as a possible surrogate for monitoring the removal of trace contaminants during wastewater ozonation. Bahr et al. (2007) showed a linear correlation between the removal of different pharmaceuticals during effluent ozonation and the decrease in the effluent's UV absorbance. The linearity was valid for ozone doses in the range of 0.2–1.5 mg O_3/mg DOC, and allowed the prediction of pharmaceutical removal based merely on absorbance measurements of the effluent.

In the present study, a linear correlation between drug removal and the decrease in SUVA was observed only up to a 10% decrease in SUVA, which corresponds to an O_3/DOC ratio of approximately 0.4 (Fig. 4). Thus, using the reduction in SUVA values to assess the ozone degradation of organic pollutants in TevaKS effluent is limited to relatively low ozone doses.

3.3.5. *Ozonation byproducts*

Although ozone has been found (here and in other studies) to efficiently remove pharmaceuticals and other micro-pollutants from water and wastewater, oxidation does not usually result in complete mineralization, but rather in the formation of oxidation byproducts. Ozonation byproducts (e.g. aldehydes, ketones, alcohols and more) can also be generated from the reaction of ozone with DOM (von Gunten, 2003).

3.3.5.1. *VLX-degradation byproducts*. Two degradation products of VLX were detected at high concentrations during the ozonation of TevaKS effluent, with molecular weights (MW) of 293.1 and 250.2. Other byproducts were most likely formed following the degradation of both VLX and CBZ; however these could not be detected due to their low concentrations and the masking effect of the effluent DOM.

Compound 1 (MW 293.1) is most likely the result of an oxygen-transfer mechanism, generating the VLX *N*-oxide (Fig. 5). It is well-established that the first step in the reaction of tertiary amines with ozone involves the addition of O_3 to the lone electron pair, followed by the release of a singlet oxygen ($^1O_2$) and the formation of *N*-oxide (Muñoz and von Sonntag, 2000). The possibility of compound 1 being the result of $HO^{\bullet}$ radical attack on the aromatic ring (Santoke et al., 2012) was ruled out, since no bathochromic shift was observed in the molecule's absorbance (typical for OH substitution on aromatic rings).

The formation of compound 1 was enhanced in the presence of 0.2 M t-butanol and supressed when pH of the effluent was reduced from 7 to 5. This behavior agrees well with a formation mechanism involves ozone reaction with an amino group. Addition of an $HO^{\bullet}$ scavenger is expected to slow the decomposition of ozone in the effluent; whereas, decreasing the pH will decrease the reaction rate of ozone with the tertiary amine. Formation of VLX *N*-oxide during ozonation is of particular interest, since this compound acts as a prodrug, which is rapidly converted in the human body back to the parent compound (Turski et al., 2010). To the best of our knowledge this is the first time VLX *N*-oxide is detected in treated wastewater effluent.

Compound 2 (MW 250.2) was previously identified by Santoke et al. (2012) as the result of an $HO^{\bullet}$ radical attack at the VLX nitrogen group. However, the formation rate of this compound was only marginally affected by the addition t-butanol or by the decrease of pH from 7 to 5. Addition of t-

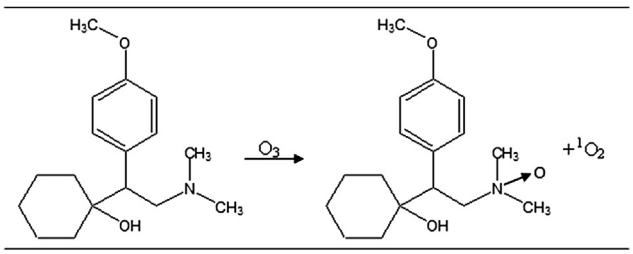

Fig. 5 – Formation path of the VLX-degradation byproducts, detected during ozonation of TevaKS effluent.

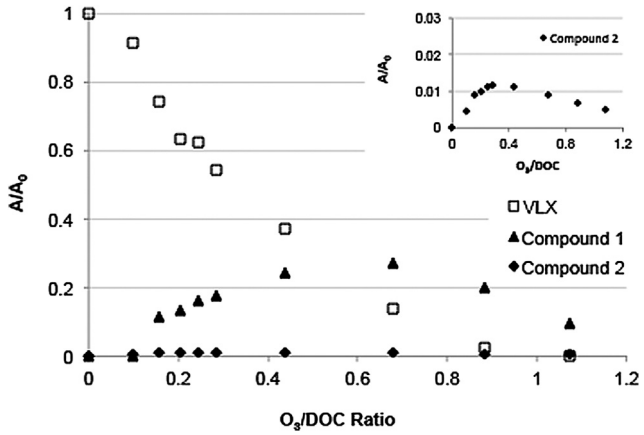

Fig. 6 – HPLC–MS peak areas for VLX and its ozonation products, normalized to the initial value of the peak area of VLX (t = 0), presented *vs.* O_3/DOC ratio. Inset is a magnification of the kinetics of compound 2 (MW 250.2).

butanol is expected to highly reduce the formation rate of compound 2, if HO• is involved. Thus, the identity of this product could not be confirmed.

Formation kinetics of the byproducts (at pH 7) is presented in Fig. 6, using their HPLC–MS peak areas normalized to the initial value of the peak area of VLX ($t = 0$ min). It is clear that degradation of the parent compound (VLX) is followed by the formation of the byproducts. Concentrations of both byproducts increase until they reach a maximum at an O_3 dose of approximately 0.7 and 0.3 (mg O_3/mg DOC) for compounds 1 and 2, respectively. Further increasing the ozone dose resulted in a decrease in the byproducts' concentration, indicating that they can be further oxidized by O_3 reactions.

Table 2 – List of compounds identified in TevaKS effluent.

No.	RT (min)	Compound name	Before ozonation	After ozonation[a]
1	6.53	Acetone	7.24 mg/L	11.01 mg/L
2	11.70	Benzene	n.d.	37.5 μg/L
3	16.88	n-Hexanal	n.d.	+
4	17.73	4,4-Dimethyl-2-pentynal	n.d.	+
5	18.84	Chlorobenzene	n.d.	42 μg/L
6	20.89	Ethyl isobutyl ketone	n.d.	+
7	22.38	DMA	+	+
8	24.59	Acetophenon	+	+
9	27.09	Bornylene	n.d.	+
10	27.47	2-(2-Furyl)-2-methyl-3-butenal	n.d.	+
11	28.01	Nonanal	n.d.	+
12	28.24	Similar to 11	n.d.	+
13	29.02	Similar to 11	n.d.	+
14	29.84	2-Methyl-1-octanol	n.d.	+
15	29.91	1-Nonanol	n.d.	+
16	30.19	6-Methyl-1-octanol	n.d.	+

+ Compound could not been quantified due to unavailability of a standard.
n.d. Not determined.
a Ozone dose of 0.8 mg O_3/mg DOC.

3.3.5.2. *Volatile organic byproducts*. Table 2 lists the main identified compounds, before and after ozonation (0.8 mg O_3/mg DOC). Sixteen compounds could be identified in the effluent after ozonation, whereas only three of them were present in the effluent before ozone was applied. Most identified compounds could not be quantified due to unavailability of standards. Acetone was by far the most abundant compound, before and after ozonation (Table 2). The presence of acetone in the effluent before ozonation may be the result of its occurrence in the untreated wastewater and its incomplete removal during the biological treatment. Acetone can additionally be formed upon ozone reactions with water and wastewater constituents (e.g. von Gunten, 2003), hence the increase in its concentration following ozonation.

Formation of volatile organic byproducts, as a result of ozone reaction with organic matter, has been previously recorded (e.g. von Gunten, 2003). Some of these byproducts are defined as priority pollutants by the EPA (e.g. benzene, chlorobenzene; EPA, 1998), emphasizing the need for a complementary biological treatment after ozonation.

4. Conclusions

- Wastewater from pharmaceutical manufacturing facilities can potentially cause significant local drug pollution due to extremely high concentration of drugs, as in the case of CBZ and VLX in TevaKS wastewater.
- A wastewater treatment train that includes biological process followed by ozonation was shown to efficiently reduce the concentrations of VLX and CBZ and can, potentially, be efficient for the elimination of other drugs.
- Using the proposed train to treat pharmaceutical wastewaters on-site (at the plant) can reduce the discharge load of drugs into the environment, while treating relatively low volume of wastewater, hence at an affordable cost. Moreover, treating these wastewaters at the manufacturing site by the manufacturer is a most desirable application of the "polluter pays" principal.
- Different ozonation byproducts were identified as a result of incomplete oxidation of the drugs and from the reaction of ozone with DOM. These byproducts are likely to be more biodegradable than the parent compounds, as suggested by the increase in the ozonated effluent's BOD_5. Therefore, a post-ozonation biological treatment is recommended.
- Ozone has decreased the effluent's specific UV absorbance-SUVA, which can be used as a surrogate to predict the elimination of CBZ and VLX (and other drugs) by ozone (at specific ozone range).

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