Pharmaceuticals, toxicity and natural fluorescence intensity of biologically treated hospital wastewater removed by pilot and laboratory scale ozonation

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Abstract We studied a bubble reactor based pilot ozonation system for removal of pharmaceuticals and toxicity from biologically treated hospital wastewater. To remove degradation products generated by ozonation, polishing with suspended biofilm carriers investigated. Removal of pharmaceuticals was comparable between the pilot treatment using a bubble column and offline laboratory experiments applying batch ozone addition. The removal rate constants of pharmaceuticals were normalized to dissolved organic carbon (DOC) and thus the efficiencies were comparable to literatures. Natural fluorescence intensity was used as an easily measurable parameter for the oxidation of organic matter in the wastewater. The remaining fluorescence after ozonation decayed slowly with holding time, but was removed fast by biofilm carriers simulating a possible polishing of ozonated effluent in a downstream biofilter. The toxicity of the hospital wastewater as measured with Microtox® and was found to reduce from 80 % to 50 % inhibition with the biological treatment. Ozonation reduced the inhibition further to 20%.

Keywords: Pharmaceuticals; Ozonation; Toxicity; Fluorescence

1. Introduction

Hospital wastewater with various pharmaceuticals is generally discharged to the sewage system ending up at a wastewater treatment plant (WWTP). However, conventional WWTPs are not able to completely degrade all pharmaceuticals and thus, some amount of refractory pharmaceuticals can still be found in the WWTP effluent (Schaar *et al*., 2010).

Moving bed biofilm reactors (MBBRs) consist of flow-through reactors containing suspended plastic carriers supporting biofilm growth. Previous researches have already demonstrated that staged MBBR treatment achieves better biodegradation efficiencies for pharmaceuticals than conventional activated sludge (Escolà Casas *et al*., 2015; Falås *et al*., 2012; Tang *et al*., 2017).

However, some of the investigated pharmaceuticals in hospital wastewater were not effectively removed even by MBBRs. Ozonation as a polishing method after biological treatment has been applied to remove pharmaceuticals in wastewater indicating that it is a promising technology for efficient degradation of these compounds (Hansen *et al*., 2016).

We investigated a pilot-scale five-stage MBBR treatment train applied for biological treatment of hospital wastewater followed by an ozone reactor remove any remaining pharmaceuticals. The biological removal of pharmaceuticals is described in a separate abstract to the conference. The aims of this work are: 1) Investigate the effect of ozone dosage on concentrations of pharmaceuticals in the effluent of the staged MBBR demonstration plant. 2) In parallel, the fluorescence intensity of ozonated effluent was studied. 3) To investigate the development of wastewater toxicity in the pilot-scale MBBR treatment train and ozonation stage. 4) To address the issue regarding of the remaining toxicity of the ozonated effluent, laboratory scale MBBR was applied to batch ozonated effluents.

2. Materials and Methods

2.1. MBBRs effluent

Wastewater from Aarhus University Hospital (Skejby, Denmark) was treated by a pilot-scale MBBR system, which consisted of a denitrification tank of 900 L (M1), a nitrification tank of 900 L (M2), a nitrification tank of 900 L (M3A and M3B), a denitrification tank of 500 L (M4) and a nitrification tank of 500 L (M5) (Fig. 1, left).

AnoxKaldnes™ K5 carriers (AnoxKaldnes, Lund, Sweden) were used in each tank with a filling ratio at 50%.

2.2. Ozone equipment at pilot and laboratory

The ozone equipment (Fig.1, left) was supplied from Air Liquide (Krefeld, Germany), and the hydraulic retention time (HRT) of reaction was13.1 min.

The ozone set-up in the laboratory was based on a 20 g/h ozone generator from O_3-Technology AB (Vellinge, Sweden), which was supplied with dry oxygen gas. The concentration of ozone stock solution was between 100 and 120 mg O_3/L.

2.3. Fluorescence and MicroTox®

Six transition pairs were used, ranging from 213 nm to 335 nm and from 310 nm to 450 nm, respectively (Hudson *et al*. (2007)). Peak A ($\lambda_{excitation,\ emission}$: $\lambda_{249,450}$), Peak B: ($\lambda_{excitation,\ emission}$: $\lambda_{231,315}$, $\lambda_{275,310}$), Peak C: ($\lambda_{excitation,\ emission}$: $\lambda_{335,450}$) and Peak T: ($\lambda_{excitation,\ emission}$: $\lambda_{231,360}$, $\lambda_{275,340}$). Peak A and C stand for the humic-like fluorophore, while peak B and T stand for protein-like fluorophore.

MicroTox® test is based on light emission (luminescence) from the marine bacterial Vibrio fischeri which is internationally recognized and standardized as ISO (2007).

2.4. Quantification

The details regarding of modified indigo method for quantification of ozone concentration conducted for this study was based on Hansen *et al*. (2016).

Detailed procedures of sample preparation and pharmaceuticals analyzed by HPLC-MS/MS, and conditions of HPLC- MS/MS instrument were based on Escolà Casas *et al*. (2015).

2.5. Quantification

To determine the ozone dosage that achieved 90% removal of each pharmaceutical in the effluent, the correlation of degradation rate of each pharmaceutical and ozone dosage was fitted by Eq. 1.

$$\frac{C}{C_0} = 10^{-(\frac{DO_3}{DDO_3})} \qquad (1)$$

Equation 1, the remaining concentration of pharmaceutical (C) is related to its initial concentration (C_0) after relevant reaction with a specific Delivered Ozone Dose (DO_3) with Decadic Dose of Ozone (DDO_3) as compound specific constant describing the required ozone dosage needed to remove 90% of the respective pharmaceutical. This based on the fact that that the decay of ozone is determined by the effluent matrix and it is independent of the pharmaceutical concentration.

3. Results and Conclusions

The concentration of the 20 investigated pharmaceuticals decreased with increasing ozone dosage and the removal in the pilot-scale ozonation was higher than in the laboratory ozone treatment when ozone concentration was less than 30 mg/L (See example in Fig 2a). To evaluate the removal efficiencies, DDO_3 for individual pharmaceutical was obtained from the curve fitting based on Eq. 1 (Fig. 2a and Table 1).

Natural fluorescence intensity monitoring is a promising tool to monitor ozonation as it is rapid and reagent free without the need for sample preparation prior to analysis which makes it suitable to be applied online. Fluorescence intensity decreased with increasing ozone dosages at both pilot and laboratory (Fig. 3a-d). The decreasing fluorescence intensity can be explained by the depletion or variation of aromatic structures and the increase of electron withdrawing groups such as –COOH in aromatic compounds (Świetlik and Sikorska, 2004; Uyguner and Bekbolet, 2005). For pilot-scale, intensity of all protein-like fluorescent peaks significantly decreased at ozone dosages around 2-10 mg O_3/L, whereas, for laboratory, intensity of all protein-like fluorescence peaks gradually decreased up to 40 mg O_3/L. In terms of investigated fluorescence wavelengths, peak T1 ($\lambda_{275,340}$, protein-like) and peak C ($\lambda_{335,450}$, humic-like) had the highest intensity compared with the rests (Fig.3. a-d). The relationship between decreasing intensity of fluorescence at several transition wavelengths and pharmaceuticals fitted a straight line (See example in Fig 2b). Therefore, it was assumed that fluorescence intensity as a surrogate has the potential ability to trace the pharmaceutical degradation and be used for online control of ozonation.

In the staged MBBR, toxicity exhibited as inhibition of bioluminescence in the Microtox assay decreased tank by tank (Fig 2c), indicating that toxicants were removed by the biofilms during the aerobic processes and the relevant removal of toxicity was attributed to the biodegradable fraction of the organic content of wastewater. The exception was the denitrifying tanks M1 and M4. Apparently the anoxic conditions in M1 and M4 cause formation of an unknown toxicant. Ozonation was able to further reduce the inhibition after MBBR.

To address the BOD formed by ozonation and the possible toxicity of ozonation by-product in ozonated effluent, a laboratory-scale MBBR was then applied as a downstream biofilter. Intensity development of fluorescence in M5 effluent, M5 effluent treated with MBBR, M5 effluent treated with ozonation and M5 effluent treated with ozonation followed by MBBR was investigated (Fig 2d). There is a clear difference in intensity between the effluent treated with additional MBBR as the biofilter and single MBBR or ozonation. Hence, ozonation followed by MBBR as a possible polishing biofilter was demonstrated that it has the ability to further reduce the fluorescence intensity. BOD removal and toxicity reduction was also shown.

Table 1. Ozone dosage for 90% removal of pharmaceuticals at pilot and laboratory and the normalization of ozone dosage to the relevant DOC condition (Z_{90}=DDO_3/DOC, DOC of this study is 40 mg O_3/L). Indicated intervals represent one standard deviation.

	Pilot		Laboratory		Z_{90}	
	DDO_3	R^2	DDO_3	R^2	Pilot	Laboratory
Ac-sulfadiazine	<LOQ[a]	<LOQ				
Atenolol	103±19	0.89	46±7	0.91	2.6±0.5	1.1±0.2
Azithromycin	<LOQ	<LOQ	<LOQ	<LOQ		
Carbamazepine	*147±53*[c]	0.63	7.4±1.1	0.96	*3.7±1.3*	0.18±0.03
Ciprofloxacin	<LOQ	<LOQ	<LOQ	<LOQ		
Clarithromycin	<LOQ	<LOQ	39±6	0.92		0.98±0.14
Diatrizoic acid	<LOQ	<LOQ	<LOQ	<LOQ		
Ibuprofen	<LOQ	<LOQ	46±8	0.78		1.1±0.2
Iohexol	151±42	0.77	*110±30*	0.69	3.8±1.0	*2.7±0.7*
Iomeprol	No fit[b]	0.41	No fit	0.44		
Iopamidol	No fit	0.01	No fit	0.34		
Iopromide	<LOQ	<LOQ				
Metoprolol	52±6	0.94	54±10	0.75	1.3±0.2	1.3±0.2
Phenazone	<LOQ	<LOQ	<LOQ	<LOQ		
Propranolol	42±9	0.79	35±2	0.96	1.0±0.2	0.88±0.06
Sotalol	<LOQ	<LOQ	<LOQ	<LOQ		
Sulfadiazine	<LOQ	<LOQ				
Sulfamethizole	50±12	0.862	<LOQ	<LOQ	1.2±0.03	
Sulfamethoxazole	56±9	0.74	*68±14*	0.66	1.4±0.2	*1.7±0.3*
Tramadol	*33±19*	0.64	31±9	0.77	*0.81±0.47*	0.8±0.2
Trimethoprim	*51±12*	0.61	29±2	0.96	*1.3±0.3*	0.73±0.06
Venlafaxine	44±7	0.90	50±9	0.77	1.1±0.2	1.2±0.2

a: If concentration was below the limit of quantification (LOQ), it indicates <LOQ.

b: If R^2 <0.5, it indicates No fit..

c: If 0.5<R^2 <0.7, it is considered as poor fit and indicates Italic.

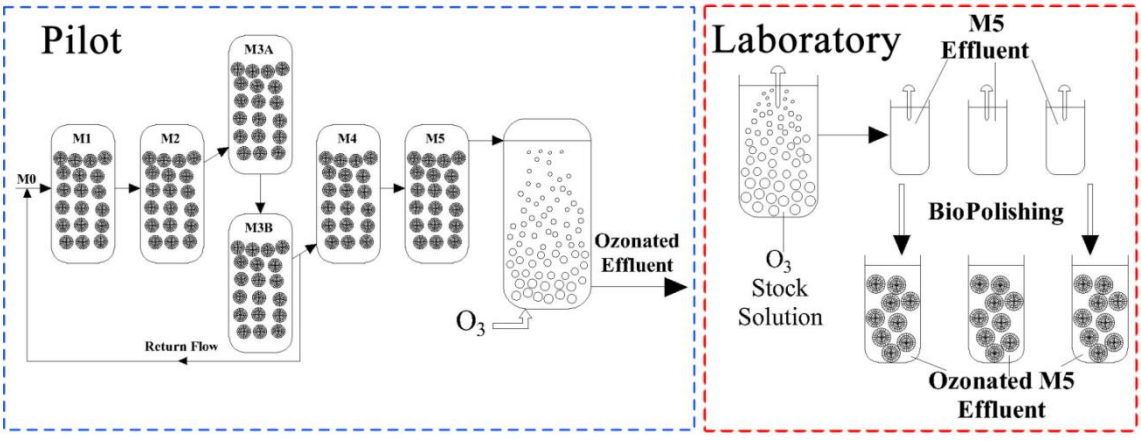

Figure 1. Schematic diagram of a five-staged pilot-scale MBBR treatment train followed by the pilot-scale ozonation (left). M0 stands for the hospital wastewater inlet. A laboratory-scale MBBR was used to polish ozonated effluent in laboratory (right).

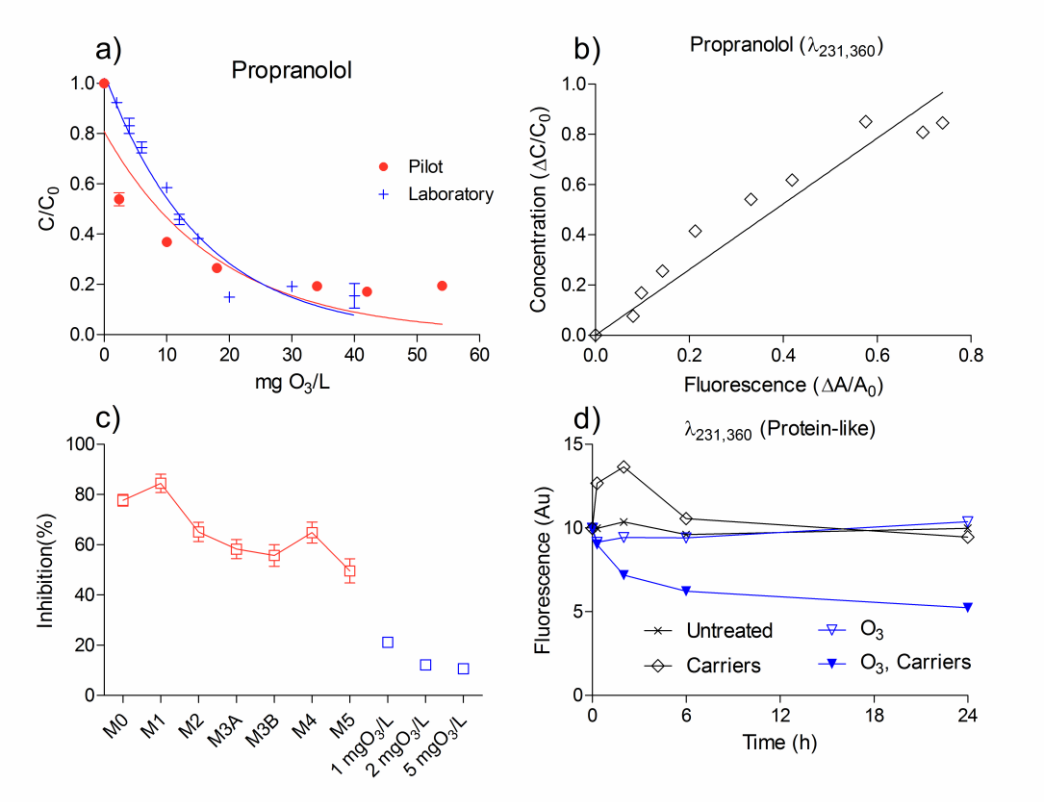

Figure 2. Comparison of removal of pharmaceuticals in pilot-scale and laboratory-scale with applied ozone doses (a); Correlation between the changes of concentrations of selected pharmaceuticals ($\Delta C/C_0$) and relative changes of excitation emission matrices fluorescence ($\Delta A/A_0$) under different dosages of ozone at laboratory (b); Toxicity development at pilot-scale MBBR and ozonation. Hospital wastewater flows through M1 reactor to M5 reactor and the flow from M2 effluent to M3A followed by M3B or firstly to M3B then followed by M3A was switched once per 12 hours. M1 and M4 are denitrification reactors while M4 has additional dosing of ethanol. However, M2, M3A/B and M5 are nitrification reactors (c); Fluorescence intensity of M5 effluent wastewater, M5 effluent wastewater with carriers, M5 effluent wastewater with ozonation and effluent wastewater with ozonation followed by carriers (d).

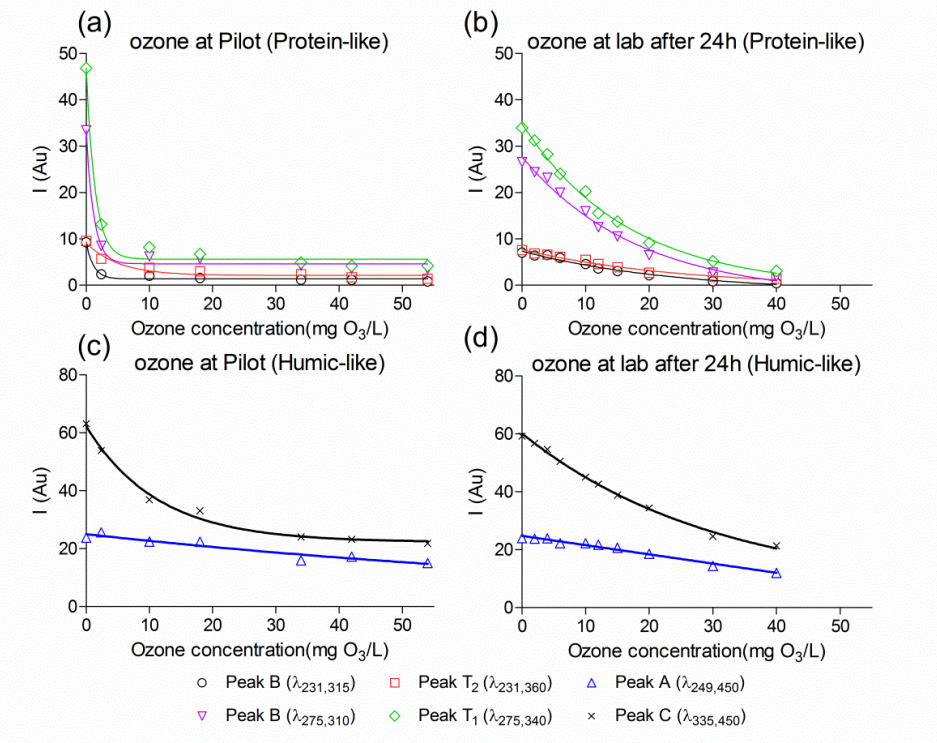

Fig. 3. (a)-(d): comparison of remaining natural fluorescence of M5 effluent treated by ozone using the pilot or laboratory method.

References

Escolà Casas, M., Chhetri, R.K., Ooi, G., Hansen, K.M.S., Litty, K., Christensson, M., Kragelund, C., Andersen, H.R. and Bester, K. (2015), Biodegradation of pharmaceuticals in hospital wastewater by staged Moving Bed Biofilm Reactors (MBBR), *Water Res* **83**, 293–302.

Falås, P., Baillon-Dhumez, A., Andersen, H.R., Ledin, A. and la Cour Jansen, J. (2012), Suspended biofilm carrier and activated sludge removal of acidic pharmaceuticals, *Water Res*, **46**, 1167–1175.

Hansen, K.M.S., Spiliotopoulou, A., Chhetri, R.K., Escolà Casas, M., Bester, K. and Andersen, H.R. (2016), Ozonation for source treatment of pharmaceuticals in hospital wastewater – Ozone lifetime and required ozone dose, *Chem. Eng. J*, **290**, 507–514.

Schaar, H., Clara, M., Gans, O. and Kreuzinger, N. (2010), Micropollutant removal during biological wastewater treatment and a subsequent ozonation step, *Environ. Pollut*, **158**, 1399–1404.

Świetlik, J., Sikorska, E. (2004), Application of fluorescence spectroscopy in the studies of natural organic matter fractions reactivity with chlorine dioxide and ozone, *Water Res*, **38**, 3791–3799.

Tang, K., Ooi, G.T.H., Litty, K., Sundmark, K., Kaarsholm, K.M.S., Sund, C., Kragelund, C., Christensson, M., Bester, K. and Andersen, H.R. (2017), Removal of pharmaceuticals in conventionally treated wastewater by a polishing moving bed biofilm reactor (MBBR) with intermittent feeding, *Bioresour. Technol*, **236**, 77–86.

Uyguner, C.S., Bekbolet, M. (2017), Evaluation of humic acid photocatalytic degradation by UV-vis and fluorescence spectroscopy, *Catal. Today*, **101**, 267–274.