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Natural fluorescence emission - an indirect measurement of applied ozone doses in polished for pharmaceuticals wastewater

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Keywords: Ozone, fluorescence, absorbance, wastewater, control, spectroscopy

Abbreviations: APIs (Active Pharmaceutical Ingredients), Dissolved organic matter (DOM), wastewater treatment plans (WWTPs), Natural organic matter (NOM), Ultra-Violet (UV) light, Dissolved organic carbon (DOC)

Abstract

This study investigated the feasibility of UV-absorbance and fluorescence as monitoring tools for ozone dosages applied to effluents from wastewater treatment plans (WWTPs). Secondary treated effluents from six Swedish WWTPs underwent ozonation (at doses ranging 0.5 - 12.0 mg O₃/L) in bench-scale experiments. Correlations between ozone dosages and UV absorbance at 254 nm and 272 nm were extrapolated with the first one being preferential for the wastewaters used because of its higher signal. UV-absorbance could detect differences in the applied ozone dosage as low as 1 mg/L which make it suitable to monitor effluent ozone treatment processes. Next, fluorescence spectroscopy was investigated at wavelength transitions that have being associated with humic-like fluorescents (Ex249Em450 and Ex335Em450) and protein-like fluorescents (Ex275Em340 and Ex231Em360 and Ex231Em315 and Ex275Em310). The Ex249Em450 transition was found to have the highest signal in all effluents and best linear regression fitting with the ozone dosages over a wide range. However, when focusing on low ozone dosages (0.5-3.0 mg O₃/L), Ex335Em450 wavelength transition showed a more constant slope among the different samples with slightly better R² values than the Ex249Em450 transition. Fluorescence removal via ozonation correlated with the pharmaceutical removal; however the wellness of fitting was directly dependent on the pharmaceuticals' reactivity with ozone. Pharmaceuticals with moderate reactivity towards ozone appeared to be linearly correlated with the Ex249Em450 transition, while very reactive or recalcitrant pharmaceuticals had an exponential or a parabolic dependency. This means that fluorescence can be potentially used as a qualitative tool for pharmaceutical removal.

1. Introduction

Wastewater effluents contain several micro-pollutants, including active pharmaceutical ingredients (APIs) and dissolved organic matter (DOM). The lack of knowledge regarding the impact of these compounds on the biotic and abiotic ecosystems is a growing environmental concern which has raised significant scientific interest to mitigate its effects [1–7]. Since conventional wastewater treatment plans (WWTPs) are unable to completely remove all APIs [8–10] and other bioactive xenobiotic micropollutants, ozone has been applied as an additional treatment step to polish wastewater effluents [3,7,11–16]. Studies have shown that the chemical structure of each API and more specifically their electronic structure determines the easiness to degrade upon ozonation [15]. In general, most APIs and miscellaneous organic micropollutants are easily degraded by low ozone dosages, ranging from 2.0 to 3.5 mg O₃/L [11,12,17]. Higher dosages are needed for the more recalcitrant to ozonation APIs, which are mostly degraded through, the secondary oxidation mechanism of ozonation, the highly reactive and non-selective hydroxyl radicals [18].

Despite the multiple benefits of ozonation (including water purification, disinfection, and detoxification, in short treatment times), ozone production is energy demanding, hence significantly increases operational cost. Thus, it is vital to deliver optimal ozone dosages continuously during ozonation operation adapting to variable water quality to achieve at minimal energy consumption both sufficient micropollutant removal and system robustness [12,14]. The key element to design an optimum ozonation system is the determination of the required ozone dosage for the specific system. However, besides the target compounds, natural organic matter (NOM) - contained in the water matrix, is also competing for ozone [13] making it harder to predict the required ozone dosage to satisfactorily remove pharmaceuticals in WWTP effluents. However, quantification the actual level of contaminants, which are usually detected in low concentrations though necessary, can be time-consuming and expensive. Therefore, it is crucial to find a way to easily correlate monitored parameters in the effluent with APIs removal as a first indication of the treatment performance.

Wastewater has discreet spectrophotometric properties of DOM with a strong light absorption in the ultra-violet (UV) region and a wide range of excitation and emission wavelengths, ranging from 200-500 nm containing both humic and protein-like fluorescent fluorephores [19–21]. When ozone is added in wastewater, the color, determined as UV absorbance at 254 nm (A_{254}) or 436 nm [22], is removed. These changes of A_{254} and/or color are correlated with the removal of several micropollutants

[23]. Nanaboina and Korshin, (2010) [24] quantified the change of absorbance spectra of ozonated wastewater using varying initial ozone concentrations. Bahr *et al.*, (2007) [12] observed a linear correlation between UV-absorbance change at 254 nm and the applied ozone dosage in WWTP effluent and the removal of miscellaneous micropollutants, while Hansen *et al.*, (2010) [14] found an equally good correlation at $\lambda = 272$ nm.

Fluorescence has occasionally been used for off- and online monitoring in water treatment applications to optimize processes [25] and to identify deteriorating agents [26]. It is able to determine rapidly and accurately DOM in aquatic environments [19,26–34] total organic carbon (TOC) [33], biological oxygen demand (BOD) [35], phosphate, nitrogen-based compounds [36] and microbial abundances [37]. A recent study suggested a method to correlate the fluorescence degradation upon ozonation with the delivered ozone dosage in recirculating aquaculture systems [38].

Although, these two spectroscopic methods appear to be promising tools to determine indirectly the applied ozone dosage due to their accuracy, immediate response, and lack of reagents, a deeper investigation is needed to determine the optimum wavelength transition that correlates fluorescence removal with the ozone dosage required to remove APIs in wastewater effluents and to establish a more solid correlation allowing for universal application of this approach. For these reasons, secondary effluents from 6 WWTPs in Sweden were spiked with a mixture of 42 APIs and treated with ozone at concentrations ranging between 0.5 -12 mg O₃/L. Then, the UV-vis absorptivity of the samples at $\lambda = 254$ nm and $\lambda = 272$ nm was recorded, followed by fluorescence spectroscopy at wavelength transitions associated with humic-like and protein-like fluorescent compounds. The obtained results were fitted with linear regression and correlated with water properties and APIs removal.

2. Material and methods

2.1. Reagents

All chemicals used in this study were purchased from Sigma Aldrich Denmark ApS and used as received. The list of pharmaceuticals that were used in the initial phase of the experiment and their suppliers can be found in Antoniou et al. [15].

2.2 Water Samples

Effluents from five WWTPs in Sweden, including Källby (Effluents 1&2), Björnstorp (Effluent 3), Öresundsverket (Effluent 4), Sjölanda (Effluent 5), and Nykvarnsverket (Effluent 6) were used in this study. Effluent 1 and Effluent 2 were from the same treatment plant but were collected on separate occasions with a 3 week time interval due to the variability of their characteristics. This difference was attributed to significant rainfall events, which occurred following the first sampling event, causing a sludge washout, reducing the biological treatment efficiency and increasing the COD value, while at the same time alkalinity value decreased because of dilution with the rainwater.

2.3 Experimental set-up

The laboratory ozone set-up was based on a 1.0 g/h ozone generator from O₃-Technology AB (Vellinge, Sweden), supplied with dry oxygen gas. The generated ozone was dispersed through a diffuser in a collection bottle containing ultra-pure water to create the ozone stock solution. To further increase ozone solubility, the bottle was submerged in an ice bath, achieving eventually a concentration of ozone in the stock solution around 30 to 40 mg O₃/L.

Effluent was spiked with the pharmaceutical standards to give a nominal concentration of 1.0 µg/L, and then transferred into borosilicate glass vials, where different volumes of ozone stock solution were added (in triplicate) to give nominal concentrations between 0.5 and 12 mg O₃/L for a total volume of 150 mL (Table S1 in SI). The dosages of ozone delivered in each experiment varied (~5% relative standard deviation, RSD) since the concentration of ozone in the stock solution was variable between days, but the delivered ozone dosage to the vials was quantified as described in section 2.4.2.

Further details of the ozonation procedure and quantification are given in Antoniou and Andersen, (2012) [39] while information on the experimental design, ozonation

treatment and pharmaceuticals removal can be retrieved from Antoniou *et al.*, (2013) [15]. The ozonated samples were eventually analyzed spectroscopically.

2.4 Quantification

2.4.1 Characterization of effluents

The characteristics of the secondary treated effluents were determined. Specifically the concentration of DOC, pH, alkalinity (mg HCO₃⁻/L), BOD₇, and ammonium-nitrogen (NH₄⁺-N) in each effluent were quantified based on standard methods.

2.4.2 Ozone quantification

The ozone concentration in water was determined as described in detail by Antoniou *et al.* [15] utilizing the indigo method [40]. The absorbance of the unreacted indigotrisulphonate was measured at 600 nm with a spectrophotometer (Hach Lange). Ozone concentration was determined by comparing the absorbance of a blank to the sample using:

$$\Delta A = A_{\text{blank},600} - A_{\text{sample},600} = 20000 \text{ L}/(\text{cm}\cdot\text{mol}) \cdot \lambda \cdot C_{\text{O}_3}$$

where λ is the path length in cm and C_{O_3} is the delivered ozone dose in mol/L.

2.4.3 Fluorescence spectroscopy

Fluorescence spectroscopy was used to determine indirectly the delivered ozone dosage in RAS water as described in Spiliotopoulou *et al.*, (2017) [38], utilizing a fluorimeter (Cary Eclipse, Varian). In natural waters, two main types of fluorophores have been detected, the humic and the protein-like components [26,27]. Several excitation/emission wavelength transitions were analyzed in the present study, representing both protein and humic-like substances contained in water. The wavelength peaks for humic substances are A (Ex₂₂₅Em₄₀₀₋₅₀₀) and C (Ex₃₀₀₋₃₅₀Em₄₀₀₋₅₀₀), while the peaks for the protein-like substances are T₁ (Ex₂₈₀Em₃₅₀) and T₂ (Ex₂₂₅Em₃₅₀) and B (Ex₂₂₅Em₃₀₅) [26,27]. However, only the wavelength transition with the highest sensitivity will be discussed in this manuscript, while results on the remaining studied wavelength transitions can be found in Supporting Information (SI).

2.4.4 Quantification of Non-volatile organic carbon

A Shimadzu ASI-V UVC/Persulfate analyzer quantified the non-volatile organic carbon of filtered samples (0.45 μm). The injected sample volume was 3.00 mL and a

calibration curve with potassium hydrogen phthalate standards from 50 to 2000 µg/L was determined ($R^2=0.9994$) with quantification limit was determined at 50 µg/L.

2.4.5 UV-vis absorbance

The UV-vis absorbance of water samples was measured directly before and after ozonation at 254 and 272 nm with a Varian CARY 50Bio UV–Vis spectrophotometer.

2.5 Data treatment

Obtained data were analyzed using MS Excel and Prism Graph Pad 5.0.

3. Results and discussion

3.1 Effluent characterization

The present study is a follow-up study of Antoniou et al. [15] where ozone was applied in wastewater effluents with variable quality to determine the dosage required to remove selected APIs. Therefore, data related to water characterization (Table 1) are the same as in Antoniou et al. [15]. The WWTPs differentiated greatly in terms of their chemical properties (including organic load, $\text{NH}_4^+\text{-N}$, and water clarity) since they received wastewater from different origin, either exclusively from household wastewater (Effluent 1, Effluent 2, and Effluent 3) or a combination of domestic and industrial wastewater (Effluent 4, Effluent 5, Effluent 6), and the implemented treatment technologies among the WWTPs differed as well. The pH was relatively stable, ranging from 6.6 to 7.2, while the water clarity, expressed as UV transmittance (UVT%) measured at 254 nm, was between 59 and 78%, which is typical for wastewater.

Table 1: Wastewater characterization [15].

	WWTP	Effluent 1	Effluent 2	Effluent 3	Effluent 4	Effluent 5	Effluent 6
Provided data		80000	80000	200	120000	300000	135000
		Domestic	Domestic	Domestic	Domestic, industrial	Domestic, industrial	Domestic, industrial
Obtained data	DOC, mg/L	7.5	6.5	5.2	8.1	13.7	8.4
	Alkalinity, mg HCO_3^-/L	244	154	185	229	256	164
	pH	6.6	6.7	7.0	7.2	6.7	6.8
	N-NH_4^+ mg/L	1.36	2.98	0.77	4.93	1.86	5.98
	A_{254} , cm^{-1}	0.206	0.190	0.104	0.171	0.257	0.168

3.2 UV Absorbance

The changes of UV absorbance with ozonation are usually measured at 254 nm (A_{254} ; [12]). However, Hansen et al. [14] observed the highest sensitivity of absorbance change at 272 nm (A_{272}) in ozonated wastewater samples. It has also been suggested that the application of differential absorbance spectroscopy (DAS) might be able to better characterize the behavior of the entire system of effluent organic matter (or NOM) chromophores [24]. Therefore, samples were analyzed in both 254 nm and 272 nm to indicate which wavelength had the highest responsiveness to ozone (Figure 1).

As expected, the UV absorbance in samples prior to ozonation (0 mg O_3 /L) varied among the WWTPs in both studied wavelengths (Figure 1), because of variation in the water matrix characteristics, source, and treatment that each wastewater effluent underwent initially. The highest absorbance was observed in the wastewater effluent from Effluent 5 (0.257 cm^{-1} at 254 nm), which received the highest volume of combined wastewater (industrial and domestic), while the lowest UV absorbance was found in the mildly organic loaded effluent (Effluent 3) (0.104 cm^{-1} at 254 nm).

Upon ozonation with approximately 7 mg O_3 / L (Figure 1), the UV absorbance in all cases decreased significantly in somewhat similar extent (remaining absorbance Effluent 1: 53%, Effluent 2: 50%, Effluent 3: 38%, Effluent 4: 44%, Effluent 5: 48%, Effluent 6: 45%). The decrease in absorptivity is due to the removal of chromophores from the effluents' organic matter [24], which resulted in an average water clarity increase of 17% (UVT%). The close 95% confidence interval on each of the determined slopes suggests that even differences below 1 mg/L in delivered ozone dosages to effluents could be detected.

Figure 1: Spectroscopic characterization of the ozonation effect on six WWTPs: a) A_{254} , b) A_{272} . T-bars indicate standard deviation; $n=3$.

In all wastewater effluents, a maximum in the difference in the UV absorbance was observed at 254 nm while the UV absorbance difference at 272 nm was slightly lower (Figure 1). A linear correlation between the absorbance removal in all wastewater effluents and the applied ozone dosage in both studied wavelengths was found (Figure 1). The A_{254} appeared to have higher sensitivity, being in agreement with Bahr *et al.*, (2007) [12], and Nanaboina and Korshin, (2010) [24], who also used the changes of A_{254} to investigate the changes of the concentration of selected micropollutants even though they insisted that this wavelength was not necessarily the optimal. Thus, a control method of ozone delivered to wastewater would rely on the determination of a response curve for each wastewater source. Variation in sensitivity for color removal in wastewater effluents over time remains to be determined. This comprises the most important lack of data required before this method can be applied for online control of ozonation. As both the loss of color of the water and the removal of micro-pollutants correlate with the ozone dosage, it is expected that the removal of the investigated compounds will also correlate with the decrease in absorbance at 254 nm as it will be discussed in section 3.3.3.

3.3 Fluorescence

3.3.1 Fluorescence characterization

Earlier studies have demonstrated the fluorescence emission spectra of wastewaters using a number of different excitation wavelengths [41,42]. This was the starting point for fluorescence to be considered as a tool for water treatment process optimization, water quality assessment, and pollution monitoring [30,33].

High fluorescence intensities were observed in wastewater samples from the six Swedish WWTP secondary treated effluents (0 mg O₃/L; Figure 2). The fluorescence intensity differentiated among the WWTPs being in line with the variation of organic matter (here described by the measured DOC) in each tested effluent. Variations in chemical and physical parameters in wastewater samples (e.g. pH, metal ion content, temperature, and suspended solids) further complicate the interpretation of fluorescence spectra [41]. The highest fluorescence intensities were observed in the Effluent 5 of the WWTP receiving both domestic and industrial wastewater while the lowest, were found in the Effluent 3 where the corresponding WWTP received solely domestic wastewater. The fluorescence intensities for the remaining WWTPs were lying in between correlating well with the organic matter content of each effluent.

In all studied WWTPs, it was clear that the highest intensities observed, had the same emission wavelength (450 nm) but different excitation wavelength (Figure 2). Both wavelength transitions, Ex249Em450 and Ex335Em450 which correspond to A and C [27], are associated with humic-like fluorescent compounds [26,27], which were dominant in the tested wastewater samples of this study and in previous studies [19,26,43]. The rest of the examined wavelength transitions, Ex275Em340 and Ex231Em360, Ex231Em315 and Ex275Em310 correspond to T and B peaks, respectively, which are protein-like fluorescent compounds [26].

Untreated wastewaters commonly consist of a broad humic-type peak C with intense T (tryptophan) and B peaks (tyrosine) [28]. Peak T generally is the most pronounced intensity peak in wastewaters [25] and it is considered a tracer and relic of anthropogenic material in natural waters [21,44]. Ishii and Boyer, (2012) [45] using Parallel Factor Analysis (PARAFAC), were able to identify a component in the same excitation and emission range as our A peak which is usually found in organic matter sources dominated by terrestrial precursor material. According to the latter [45], C peak was defined as reduced quinone-like compounds, identified in organic matter from a wide variety of aquatic systems, including those dominated by terrestrial and microbial

inputs. Humic-like fluorophores have been found to dominate in wastewater, municipal wastewater [33] and recirculating aquaculture studies [38]. Peak A and Peak C result from the presence of both carbon-carbon double bonds and aromatic carbon bonds in fulvic acid-like and/or humic-acid-like components [43].

Carstea *et al.*, [33] studied the removal of various fluorescent compounds during wastewater treatment and compared components associated with peaks T and B with peaks A and C. It was reported that both A and C peaks were removed to a lower extent in the first stages of the treatment plants compared to peaks B and T. Peak B was readily reduced in the anaerobic zone and was extinct after the oxic zone. Biological treatment had an extensive impact on peak T removal (up to 80%); while a moderate effect on peak C (10-30%) was observed. Tertiary treatment (e.g. filtration, stabilization ponds etc.) and the subsequent polishing step (e.g. UV or chlorination) were able to further remove both peaks. By the end of the treatment, peak T and in general peaks with $E_m < 380\text{nm}$ exhibited great removal during conventional treatment plans [26], which was also observed in this study.

In previous studies the humic-like peak C was found in higher intensities in the effluent compared to peak A due to the fact that these products are generated by substrate utilization or biomass decay and cell lysis, and are regarded as autochthonous matter [46]. However, in all studied wastewaters peak A was found in higher intensities than peak B. Cohen *et al.*, (2014) [47] and Riopel *et al.*, (2014) [31] suggest that these fluorescent components are either potentially produced during the process or are recalcitrant to decomposition. Riopel *et al.*, (2014) [31] also suggested that large molecules degrade into smaller molecules that have a fulvic-like behavior, based on the polyphenol postulate of humic substances formation [31].

Figure 2: Fluorescence removal measured in six wavelength transitions [26] due to ozonation of six wastewaters. T-bars indicate standard deviation; n=3.

3.3.1.1 Optimal wavelength transition

Although the analysis of the samples with the six different wavelength transitions was useful to characterize the untreated with ozone wastewater, a deeper investigation regarding the sensitivity of each excitation/emission wavelength to ozone is needed.

All studied wavelength transitions (Figure 2) were analyzed individually and relevant data can be found in supplementary information (Figure S1 – Figure S6). Among them (Figure S1 – Figure S6), the highest signal in all wastewater samples was found at the Ex249Em450 (Figure 3). In recirculating aquaculture samples, the wavelength transition Ex335Em450 had the highest signal [38]. Baker *et al.*, (2004) [36] on the contrary, found that the tryptophan-like fluorescence (Ex231Em360), which is highly correlated to human pollution, had the highest signal in river and wastewater.

Ozonation had a substantial impact on fluorescence intensity. Results suggest a good correspondence of fluorescence removal with the applied ozone dosages, expressed with the first order decay relation (Eq. 1), achieving high R^2 in all cases.

$$Y = (Y_0 - \text{Plateau}) * \exp^{(-k * X)} + \text{Plateau} \quad (\text{Equation 1})$$

where, X: ozone dosage (mg/L); Y: starts at Y_0 and decays (with one phase) down to Plateau; Y_0 and Plateau: Same units as Y (AU); k: rate constant (L/mg).

Ozonation was able to remove significantly the fluorescence intensity of the wavelength transition Ex249Em450 in all wastewater samples. Even at low ozone dosages (less than 5 mg O_3 /L) fluorescence intensity reduced up to 50% (Figure 3). Similar findings have been reported in recent studies where fluorescence sensitivity towards ozone had been investigated in ozonated samples from intense recirculating aquaculture systems [38].

Figure 3: The effect of fluorescence removal measured in the wavelength transition Ex249Em450 due to ozonation of six wastewaters. T-bars indicate standard deviation; $n=3$.

3.3.1.2 Increased sensitivity

The concept of an ozone sensor based on fluorescence reduction because of ozonation has already been suggested in recirculating aquaculture systems while the importance of a calibration curve (linear regression) and the need for a deeper investigation in terms of range and slope sensitivity have been also stressed in a recent study [38]. Therefore, the data shown in Figure 3 were fitted with the linear regression function (Figure 4), which found to have the best fit at Ex249Em450 compared with the remaining wavelength transitions (Figures S7-S12; Table S2-S3).

Figure 4: Fluorescence removal measured a) at the wavelength transition Ex249Em450 following ozonation of the six effluents (complete ozonation range), fitted with linear regression and b) at the wavelength transitions Ex249Em450 and Ex335Em450 following ozonation of the six effluents in the low ozonation range. T-bars indicate standard deviation; n=3.

Similar to the one phase decay fitting, the high correlation between fluorescence removal and applied ozone dosage was also present in the linear regression fitting. The slope of the curve and the range are crucial components used to define the calibration properties [38]. To ensure that the Ex249Em450 was the optimal wavelength transition, the slopes and the coefficient of determinations were compared (Table S2), proving that indeed it was more accurate (higher R²) and with higher sensitivity (the bigger the slope, the more profound the change when adding 1 mg O₃/L) than the EX335Em450 in the wide ozonation range (0-11 mg O₃/L).

According to the cited literature, an ozone dosage of 2.0 to 3.5 mg O₃/L [11,12,14] is able to degrade several APIs and micropollutants in water. Thus, the results in Figure 4 were analyzed in a lower range (0-3 mg O₃/L) to investigate the sensitivity of fluorescence. Based on Figures 2-4, it is clear that the wavelength transition with Ex249Em450 can adequately describe the fluorescence removal upon ozonation of wastewater effluents. However, when focusing on the low ozonation range, the comparison between the Ex249Em450 and Ex335Em450 (Table S3) revealed

that the R^2 of Ex249Em450 was remarkably lower than when looking at the whole ozonation range (Table S2) and that the wavelength transition provided a more constant slope among the different samples with slightly better R^2 values than the Ex249Em450. Therefore, it appears that the wavelength transition with the highest signal is not necessarily the most sensitive for low ozone dosage range. This needs to be taken in consideration when applying this methodology in the field for continues ozone monitoring.

3.3.2 Correlation of fluorescence with water quality parameters

It has been proven that fluorescence is highly correlated with several effluent matrix parameters such as DOM, TOC [27,31,49], BOD [25,33,43,49] phosphate, nitrogen-based compounds [36,43] and microbial abundances [37]. Currently, there are commercially available devices convert the peak T fluorescence signal into BOD equivalent values, using an internal calibration factor or a multispectral approach [50,51].

The UV absorbance at 254 nm (ABS254) and DOC of non-ozonated water samples (0 mg O₃/L) from the six WWTPs were plotted against the initial fluorescence (Figure S18). Although Pearson analysis suggests that there is a correlation between the fluorescence with each one of the studied parameters (Figure 5), more data points are needed to better support this statement. In contrast to Hur and Cho, (2012) [43] findings for river water, the humic-like fluorescent components in the effluents appear to have higher correlation with the studied parameters compared with protein-like components (Figure S19). However, previous studies have reported poor correlations ($R^2 < 0.4$) between DOC and TOC in a wastewater treatment plant (Ex280Em300–450) [52], while more accurate prediction of DOC from fluorescence ($R^2 > 0.9$) was documented by Marhaba *et al.*, (2003) [53], who used a three-component PCR model developed from 69 EEMs (Ex225–500Em231–633) collected from a canal supplying various New Jersey water treatment utilities.

Reported correlations of Pearson's coefficient for peak T (Ex270Em340) and the DOC values for wastewaters typically range between 0.42 and 0.97 [52,54,55]. The wide range of correlations that have been observed for fluorescence (especially peak T) and DOC are explained by the ratio of refractory DOM to labile DOM, of fluorescent and non-fluorescent character. Based on our individual estimation indices showing the highest correlation coefficients with ABS254, COD and DOC, the regression equations

are suggested as $ABS_{254} = 0.422 \times 10^{-3} \times \text{Fluorescence} - 0.36 \times 10^{-2}$ and $DOC = 0.0256 \times \text{Fluorescence} - 3.662$, respectively (Figure 5).

Figure 5: Correlation between fluorescence (Ex249Em450) and the absorbance at 254nm and DOC in the six non-ozonated wastewater effluents.

3.3.3 Correlation of fluorescence with pharmaceutical removal

In a previous publication by Antoniou *et al.*, (2011), 42 APIs were monitored for their recalcitrance to ozonation in the six effluents. It was reported that irrespectively of the matrix of each effluent; APIs can be categorized into easily degradable, moderately degradable and persistent to ozonation. With ozonation affecting the fluorescence of the samples it was imperative to investigate whether and how APIs removal correlates with fluorescence removal per delivered O_3 dose. For this reason, representative APIs from each category were selected and plotted against fluorescence which had the highest signal (Ex249Em450nm) for each one of the effluents. Figure 6 depicts the remaining concentration of APIs per ozone dose with the remaining fluorescence at Ex249Em450nm of Effluent 1 (Kalby 1). The correlation of the remaining effluents can be found in SI (Fig. S13-S17, Table S4-S9). By doing so, it became apparent that API removal followed a different trend depending on the category of ozone reactivity each API was assigned to, irrespectively of the matrix characterizes of the effluent tested. Easily degradable with O_3 APIs, followed an exponential trend, moderate degradable with O_3 APIs followed a linear trend while persistent APIs could either follow a linear, parabolic, or scattered trend. Specifically, the easily degradable APIs were being removed much faster than the remaining matrix components that were still giving fluorescence signal, hence the exponential decay. The moderate APIs ($0.75 < [DDO_3 / DOC] < 1.3$) degraded with the same rate as the matrix components (linear

correlations), while for the trend for the recalcitrant APIs was more specific towards the compound treated. Based on the above, it can be concluded that fluorescence removal can be used as a qualitative tool to refer to APIs removal from ozonated samples, when the content in APIs is already known.

Figure 6: Correlation of matrix (Effluent 1; Kalby 1) fluorescence at Ex249Em450nm with the corresponding remaining API concentration

3.4 Online monitoring of ozone dosage in WWTPs

The fluorescent properties of organic matter have attracted the attention of scientists over the years [33,56] and the online monitoring of fluorescence in treatment plants has been already suggested [30,42,49,57]. Given the high sensitivity of fluorescence to

ozone, we believe that fluorescence could be used as an online tool to control the ozone dosage in full scale WWTPs (Scheme 1). This concept has been previously suggested by Spiliotopoulou *et al.*, (2017) [38] to control ozonation in recirculating aquaculture systems. A calibration curve from the specific WWTP is required in order to characterize the wastewater effluent. A fluorescence intensity within the calibration curve will be chosen as a control point (e.g. 450 AU, as illustrated in Scheme 1), and a predetermined fluctuation would be accepted. When fluorescence intensity goes beyond that range, ozone will be dosed accordingly in order to maintain the fluorescence within the set limits.

Although, the results revealed that the fluorescent compounds did not have the same response, the filter-based *in situ* fluorometers are able to collect fluorescence signals over a range of wavelengths, which should improve the effect of slight variations in either the optical parameters of the instrument or the fluorescence characteristics of samples [57]. With this approach, the actual applied ozone dosage will be determined ensuring an efficient and robust treatment.

Spiliotopoulou *et al.* [38] have also introduced the concept of offline monitoring of ozone (without any sensor installation) in recirculating aquaculture systems which could be also applied in WWTPs. In brief, the ozone quantification could be achieved by collecting water samples before and after ozonation is applied, transfer them to the laboratory and then create an ozone calibration curve with the untreated effluent. Based on the calibration curve, the actual ozone dosage in the water can be read as fluorescence for that specific system and then be directly converted to ozone concentration. This approach allows for checking periodically the performance of the ozone generator and comparing the obtained data with the manufacturer's specifications, thus ensuring the optimal operation of the equipment, which consequently has a monetary value [38]. It can also be applied in the case where experiments are performed in remote locations and a significant number of samples are collected in a short period. Based on this approach, the samples can be stored in a fridge and quantified later.

Scheme 1: Schematic representation of the application.

4. Conclusions

The significance of the correlation between the removal of pharmaceuticals with UV-absorbance and fluorescence was investigated in water samples from 6 WWTPs. Results suggest that fluorescence can potentially be used as a method to monitor the applied ozone dosages to remove a great variety of pharmaceuticals in WWTPs. This study revealed that the wavelength transition Ex249Em450 is the most suitable transition for ozone monitoring in WWTPs. However, when focusing on the low ozonation range, the comparison between the Ex249Em450 and Ex335Em450 revealed that the latter had a more constant slope among the different samples with slightly better R^2 values, which should be taken into consideration when this methodology is applied in the field for continuous monitoring. Fluorescence removal via ozonation correlated with APIs removal, though their susceptibility to ozonation affected the wellness of correlation. In any case it can potentially be used as a qualitative tool for APIs' removal. To conclude, previous works as well as our investigations have indicated strong correlations between traditional wastewater quality parameters and fluorescence, although there are still issues when directly comparing fluorescence data between different sample sources.

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