Coupling of nanofiltration and UV, UV/TiO_2 and UV/H_2O_2 processes for the removal of anti-cancer drugs from real secondary wastewater effluent

R. Janssens[a], M. B. Cristovao[b], M. R. Bronze[b,c,e], J.G. Crespo[d], V. J. Pereira[b,e], P. Luis[a]

[a]Materials Process Engineering (iMMC-IMAP), Universite catholique de Louvain, Place Sainte Barbe 2, 1348 Louvain-la-Neuve, Belgium

[b]iBET-Instituto de Biologia Experimental e Tecnologica, Oeiras, Portugal

[c]iMED, Faculdade de Farmacia Universidade de Lisboa, Lisboa, Portugal

[d]LAQV-REQUIMTE/Department of Chemistry, Faculdade de Ciencias e Tecnologia, Universidade Nova de Lisboa, Caparica, Portugal

[e] Instituto de Tecnologia Quimica e Biologica Antonio Xavier, Universidade Nova de Lisboa, Oeiras, Portugal

*corresponding author: raphael.janssens@uclouvain.be

Highlights

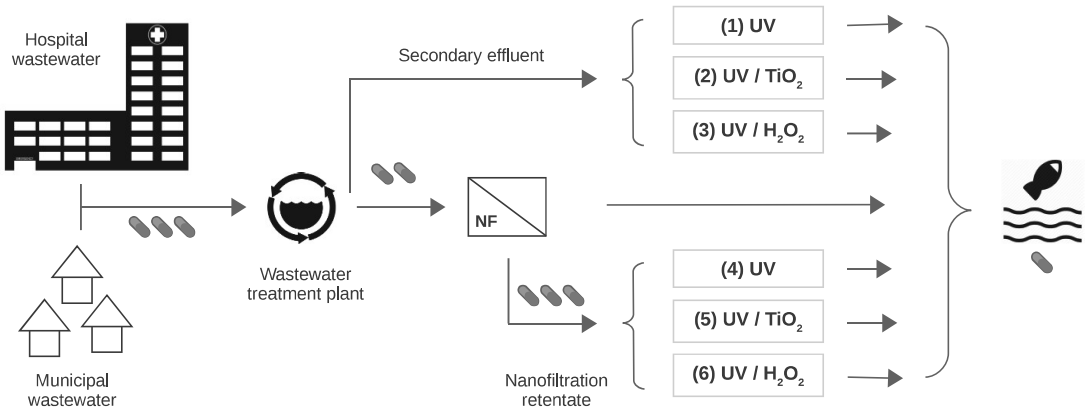
- Aggregation of TiO_2 in real matrix hinders photocatalytic formation of OH radicals
- Complete degradation of ETP and PAC in secondary effluent by direct photolysis
- Complete degradation of ETP and PAC in nanofiltration retentate by direct photolysis
- CP and IF were not degraded using the conditions tested

Keywords

Photolysis; advanced oxidation processes; anti-cancer drugs degradation; secondary effluent; nanofiltration retentate treatment

Abstract : The detection of anti-cancer drugs in surface waters at ng/L indicates that most wastewater treatment processes currently applied are not effective enough to remove these resilient compounds from wastewater. Therefore alternative treatment processes should be tested to avoid discharges in the environment of drugs that have been linked to genotoxic effects. The innovative contribution of this study stands in coupling nanofiltration with advanced oxidation processes to treat real secondary wastewater effluents spiked with 4 widely consumed anti-cancer drugs. Direct photolysis was found to be extremely effective to degrade etoposide and paclitaxel from the secondary effluent as well as from the highly concentrated retentate produced by nanofiltration. The two drugs were not detected after 10 minutes of exposure that corresponds to higher than 98% removals given the method detection limits by direct injection. This is equivalent to pseudo-first order degradation rate constants higher than 0.46 min^{-1}, which, to the best of the author's knowledge, has not yet been reported for paclitaxel in the literature. However, none of the tested oxidation processes (UV, UV/TiO_2 and UV/H_2O_2) using the reactor setup, exposure times and concentrations of TiO_2 and H_2O_2 tested, were able to degrade cyclophosphamide or ifosfamide effectively. Compared to literature data, this is probably related to the low amount of photons received by the treated media. Finally important aggregation of catalyst particles was observed in secondary effluent, and photocatalysis had no advantage compared to the other two processes tested.

Graphical abstract



1 Introduction

Anti-cancer drugs, also called cytostatic drugs have been detected in surface waters at ng/L levels (Booker et al., 2014). As these emerging contaminants are genotoxic, mutagenic and fetotoxic for human (Kummerer et al., 2000), negative impacts on natural ecosystems is likely to result from long time exposure : Novak et al. showed that a mixture of four drugs exerted low cytotoxicity towards zebrafish liver cells, however it induced significant DNA stand break formation at relevant environmental concentrations of cyclophosphamide, ifosfamide, fluorouracil, cisplatin (12, 10, 0.09 and 0.6 $\mu g/L$, respectively) (Novak et al., 2017). Therefore prioritization lists based on state of the art of toxicity data were realized by various groups of research in France, Portugal, Spain and United-Kingdom (Besse et al., 2012; Booker et al., 2014; Escudero-Onate et al., 2017; Franquet-Griell et al., 2015; Toolaram et al., 2014). The four cytostatic drugs investigated in this work were selected from these lists taking into account environmental risk quotients, diversity of molecular structures and chemical properties: paclitaxel, etoposide, cyclophosphamide and ifosfamide. In addition to be toxic, some anti-cancer agents are designed to be chemically stable in order to maximize probabilities of reaching the body parts where therapeutic effects are required (Sanderson et al., 2004). Consequently some cytostatic compounds are non-biodegradable (Kummerer et al., 2000) and biological treatments applied in

conventional wastewater facilities are not effective for removing these emerging contaminants.

In last decades, nanofiltration (NF) modules were implemented in treatment stations as effective barriers against discharge of micro-pollutants in surface waters (Radjenović et al., 2008; Taheran et al., 2016). Zhao et al. reported mean rejections above 90% of 23 pharmaceuticals spiked in deionized water with NF membranes operating at lower pressures than reverse osmosis modules (Zhao et al., 2017) However the influence of water composition on separation properties of membranes has been reported by earlier studies (Boedec et al., 2017; Kimura et al., 2009). A recent study conducted by our team compared the potential of two nanofiltration membranes (Desal $5DK$ and $NF270$) to remove paclitaxel, etoposide, cyclophosphamide and ifosfamide) from different matrices (laboratory grade water, synthetic urine and real secondary effluent). Desal $5DK$ proved to be more effective than $NF270$ to remove the target drugs from the different matrices and proved to be less susceptible to fouling in the experiments performed with real secondary effluent (Cristóvão et al., 2019). However the concentrated retentate was found to be toxic to freshwater crustaceans and should therefore be subject to further treatment.

Advanced oxidation processes are promising technologies for post-NF treatment (Joo and Tansel, 2015) as high removal efficiencies of pharmaceuticals were reported by ozonation, photocatalysis and chemical oxidation due to the formation of free radicals having strong oxidizing power (Koltsakidou et al., 2017; Lin and Lin, 2014; Fiszka Borzyszkowska et al., 2016; Lai et al., 2015; Ofiarska et al., 2016; Molinari et al., 2008; Janssens et al., 2017). The aim of this work was to assess performances efficiencies of UV, UV/TiO_2 and UV/H_2O_2 to degrade anti-cancer drugs from two different matrices: secondary wastewater effluent and nanofiltration concentrate. The novelty of the present work is multiple: i) regarding the effect of real matrix on photocatalytic formation of OH radicals, ii) regarding the comparison of the effectiveness of different treatment processes (nanofiltration and direct or indirect photolysis) to treat widely used anti cancer drugs and iii) regarding the evaluation of using direct and indirect photolysis to treat a highly concentrated retentate.

2 Material and methods

2.1 Materials and handling precautions

Paclitaxel, etoposide, cyclophosphamide, ifosfamide were purchased at the highest purity grade ($> 98\,\%$) from Sigma-Aldrich, Germany (their molecular structures are represented in Figure 1. Stock solutions were prepared in methanol following safety guidelines reported for cytostatic drugs handling (Eitel et al., 1999). Samples and solutions were stored in the dark at $-20°C$ as recommended by Negreira et al. (Negreira et al., 2014). Presence of free hydroxyl radical in solution was indirectly assessed using a molecular probe - para-chlorobenzoic acid (pCBA) - described by Elovitz and von Gunten (Elovitz and Von Gunten, 1999) and purchased from Sigma-Aldrich, Germany, at highest purity grade favailable ($> 98\,\%$). Titanium dioxide Degussa P25, a mixture of $70\,\%$ anatase and $30\,\%$ rutile (Evonik Degussa, Germany) was used for the oxidation experiments as well as hydrogen peroxide purchased from Sigma-Aldrich, Germany ($30 - wt\,\%$ in H_2O).

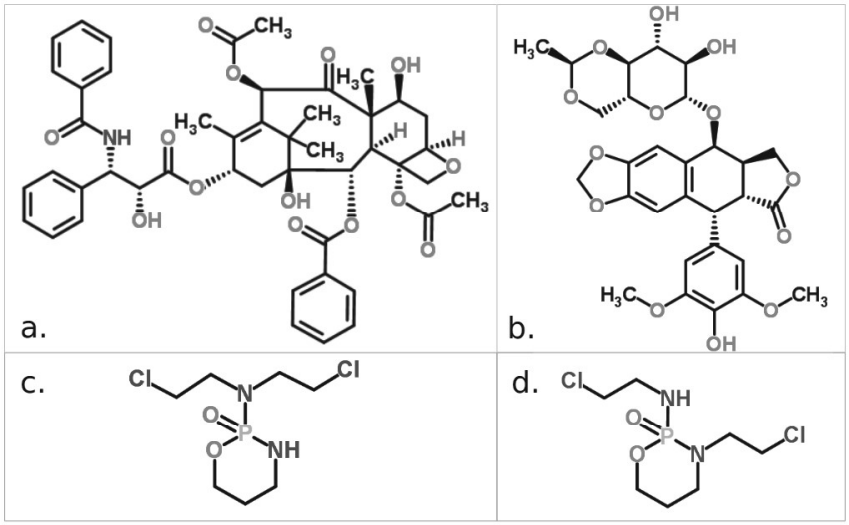


Figure 1: Molecular structures of a) paclitaxel, b) etoposide, c) cyclophosphamide and d) ifosfamide drawn on ChemSpider *www.chemSpider.com*

2.2 Water matrices

Two water matrices were investigated in this work. The first one was laboratory grade water produced by a MilliQ water system (Millipore, CA, USA; Resistivity:18.2 $M\Omega$ at $25°C$; TOC$< 5\mu g/L$; bacteria$<$ 1 CFU $/mL$; particulates $< 0.22\,\mu m$). The second one was a real secondary wastewater effluent collected at the biological treatment outlet of a municipal wastewater treatment plant located in Lisbon, Portugal. The grab sample collected in January 2018 was stored in the dark at $4°C$ in glass bottles and tested within 10 days after sampling. Characterization of this effluent was published in a previous work (Cristóvão et al., 2019) and is available in supplementary data in Table S1.

2.3 Oxidation experiment

Degradation tests were performed on four anti-cancer drugs: etoposide (ETP), paclitaxel (PAC), cyclophosphamide (CP) and ifosfamide (IF). In European wastewaters, anti-cancer agents were detected at maximum concentrations of 124 $\mu g/L$ for 5-fluorouracil (Mahnik, 2007) and 100 $\mu g/L$ for carboplatin (Lenz et al., 2007). In order to reflect real occurrence levels the four drugs were spiked in the secondary effluent at concentrations of similar order of magnitude to those detected in wastewaters: 500 $\mu g/L$ individually. This concentration permitted to observe degradation rates taking into account detection limits (about 10 $\mu g/L$) by direct injection in the HPLC-MS/MS using selective ion monitoring.

Degradation tests were performed in a collimated beam reactor with a 600W medium pressure mercury lamp (UVH 1019 Z-6 from UV-Technick Meyer gmbh). Experiments were conducted at room temperature using double walled petri dishes and the spiked solution was continuously stirred. At regular intervals, a shutter was used to block UV rays while 1 ml samples were taken from the 50 ml photoreactor. Personal protection against armful UV light was also used (complete skin coverage with laboratory coat and anti-UV face masks). As cellulose acetate

was reported to be a low absorbent material towards anti-cancer drugs (Negreira et al., 2014), cellulose acetate syringe filters (0.2 μm) were used to prevent suspended catalyst particles and natural organic matter to be injected in the HPLC-MS/MS equipment.

Tests on pCBA lasted 1h while cytostatic experiments were performed for 3h. In parallel of each experiment, samples in dark conditions evidenced that no adsorption or degradation of interest compound occurred. Reproducibility of pCBA degradation was assessed by duplicate experiments. However essays involving cytostatic drug handling were not repeated in order to reduce exposure to armful compounds. In this case, toxic waste was disposed as hazardous special waste. Production of hazardous liquid waste was reduced thanks to mindful cleaning and reuse practices and the use of small reactors.

2.4 Membrane filtration

As presented in Equation 1 the permeability of the investigated Desal 5 Dk nanofiltration membrane (MWCO 150-300 Da, GE Osmonics, USA) was calculated by dividing the transmembrane flux (J) by the transmembrane pressure (TMP) and the active area of filtration (A). During preliminary tests, a laboratory grade water permeability of 3.38 Âś 0.07 $L/(h.m^2.bar)$ was measured.

$$P = \frac{1}{A}\frac{J}{TMP} \tag{1}$$

In order to produce the required amount of retentate for oxidation tests, several batch filtrations were carried out with the dead-end filtration module. One batch test corresponds to the filtration of 250 ml of feed solution (V_f) at 10 bars until 50 ml remains at the retentate side (V_r) and 200 ml at the permeate side (V_p). Retentate volumes were collected, mixed together and stored in dark at $4°C$. More details on the filtration procedures used can be found in a previous publication (Cristóvão et al., 2019).

Drug concentrations in feed, retentate and permeate samples were quantified by HPLC-MS/MS (C_f, C_r and C_p) and membrane rejection percentages (R) for each anti-cancer drugs were calculated by $R = (C_f - C_p)/C_f * 100$. In addition the percentage of cytostatic compounds adsorbed on membrane was assessed by the following mass balance:

$$ads_{\%} = \frac{C_f * V_f - C_r * V_r - C_p * V_p}{C_f * V_f} * 100 \tag{2}$$

2.5 Analytical methods

Compounds of interest were quantified by HPLC-UV and HPLC-MS/MS with a Luna C18 separation column (5 μm, 150 x 2.0 mm) thermostatted at $35°C$. For pCBA analysis, 10 μL of sample were injected through the column at 0.8 ml/min in an isocratic mode using a mobile phase of 50/50-water/acetonitrile. The molecular probe was quantified with an UV absorbance detector operating at 240 nm wavelength. Anti-cancer drugs were separated using the same column but with a solvent gradient presented in Table 1. For drugs quantifications, the column was connected to a HPLC-MS/MS detector (Waters Alliance 2695 module, Ireland). More details on these analytical methods were published in a previous work (Cristóvão et al., 2019).

Table 1: Solvent Gradient for HPLC-MS/MS

Time (min)	0	7	10	12	15
Solvent B %	5	100	100	5	5

A: 0.5 % formic acid in Milli-Q
B: acetonitrile

3 Results and discussions

3.1 Catalyst load optimization

Para-chlorobenzoic acid (pCBA) can be used as molecular probe to assess hydroxyl radicals formation during photocatalytic reactions. This methodology, proposed by Elovitz and von Gunten (Elovitz and Von Gunten, 1999), has been applied in previous researches to indirectly quantify hydroxyl radicals formation (Brunet et al., 2009; Rosenfeldt et al., 2006; Pereira et al., 2007). In the present work, degradation tests were performed at different catalyst loads and experiments inducing the fastest degradations were interpreted as experiments with the highest OH radical production. As presented in Figure 2, the linearity of $ln(C/C_0) = -k * t$ regressions indicate that pCBA degradation followed a pseudo-first order kinetics ($R^2 > 0.95$). The slopes of these regressions, called time-based apparent degradation rate constants (k), measured in laboratory grade water and secondary wastewater effluents using different TiO_2 concentrations were reported on Figure 3. The trend of removal rates showed in Figure 3 depends strongly on the matrix investigated.

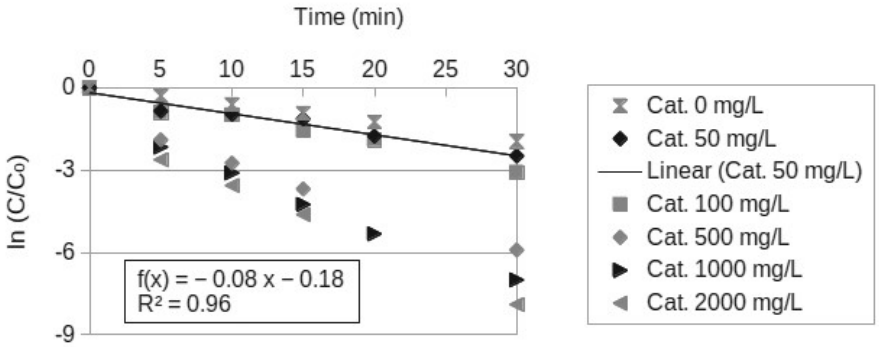


Figure 2: Pseudo-first order kinetics of pCBA (5 μM) degradation at various catalyst loads in laboratory grade water

In absence of catalyst, direct photolysis of pCBA occurred in the two water matrices. This

is probably related to the emission of powerful photons by the mercury lamp which are able to break pCBA stable covalent bond. The addition of catalyst in suspension had a significant impact on pCBA degradation rates as presented in Figure 3. In laboratory grade water, degradation rates first increased with TiO_2 concentrations and then flattened at high catalyst load (2000 mg/L) because the high particle concentration reduces the light penetration length. A similar trend was obtained in our previous work on photocatalysis using LEDs as light source (Janssens et al.). This effect is defined in literature as light screening effect (Umar and Aziz, 2012). In real effluent, a similar increasing trend was observed for catalyst concentrations below 100 mg/L. However above a TiO_2 concentration of 500 mg/L removal rates decreased to become negligible.

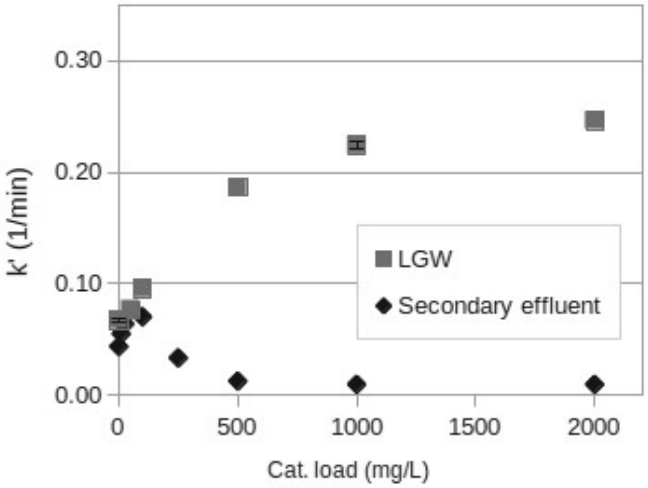


Figure 3: Catalyst load optimization based on the pCBA degradation time-based degradation rate constant obtained at different TiO_2 concentrations in laboratory grade water (squares) and secondary wastewater effluent (diamonds)

Microscopic pictures of the catalyst slurry were taken at 100x magnification in samples containing 50 and 500 mg/L of TiO_2 dispersed in the two investigated matrices: laboratory grade water (LGW) and secondary effluent. Homogeneous particle distribution was assessed and verified by scanning various areas of each sample. Microscopy pictures presented in Figure 4 help to understand what happens with the catalyst in secondary effluents. In the first set of pictures

realized in laboratory grade water (see Figures 4.a-b), aggregates of similar sizes were identified increasing in numbers with the catalyst load. In the second set of pictures taken in secondary water (see Figures 4.c-d), agglomerates of larger sizes were observed having islands-like shape instead of small homogeneous white dots. Nanoparticles aggregation depends on the electrostatic state of the double layer formed by various species in solution. If electrostatic repulsion between particles, called zeta-potential, deviates from zero, individual catalyst particles having nominal sizes of 25 nm will tend to aggregates (Topuz et al., 2015). Ottofuelling et al. have evidenced that the presence of organic matter in natural water favours TiO_2 dispersion, whereas divalent ions (e.g. Ca^{2+}, SO_4^{2-}) elevate zeta-potential leading to the formation of micrometer size aggregates (Ottofuelling et al., 2011). In regards to the number and concentration of divalent ions present in the secondary effluent investigated (see Table S1), it is not surprising to observe aggregates of micrometer scale in the real matrix. The decrease of pCBA degradation rates observed in Figure 3, might be explained by the presence of these aggregates seen in Figures 4.c-d: above a certain catalyst concentration (100 mg/L) light screening effects become more and more significant until the formation of OH radicals is completely inhibited. Therefore 100 mg/L was selected as optimal catalyst load for further experiments conducted in real wastewater effluents.

3.2 Optimization of photo-chemical oxidation

Besides photocatalytic essays, chemical oxidation tests were performed with hydrogen peroxide diluted in the secondary effluent (20 - 150 mg/L). The evolution of pCBA concentrations during experiments indicated that degradation kinetics followed a pseudo-first order ($ln(C/C_0) = -k*t$). As presented in Figure 5, under UV irradiation and without hydrogen peroxide, limited pCBA degradation was obtained. However a linear increase of the time-based degradation rates was observed with the amount of H_2O_2 added in solution. In contrary to photocatalytic results in laboratory grade water, chemical oxidation showed no limitation to the amount of hydrogen

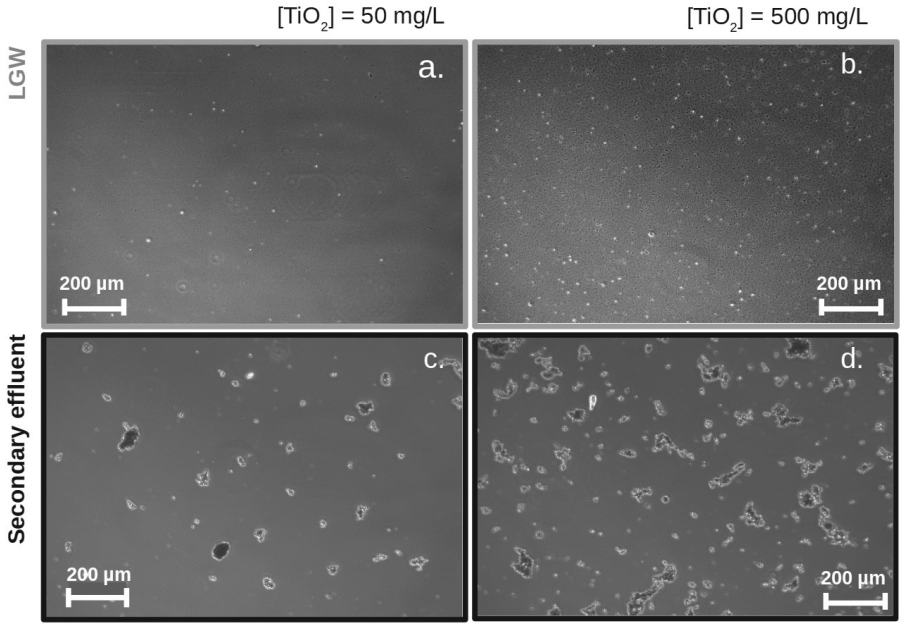


Figure 4: Optical microscope pictures (100x magnification) of different catalyst concentrations dispersed in laboratory grade water (a-b.) and secondary effluents (c-d.)

peroxide used. Therefore, in order to select optimal oxidant concentration, economic aspects were taken into account. Results of an economic analysis comparing UV/H_2O_2, $UV/S_2O_8^{2-}$ and solar photo-fenton at pilot scale indicated that 40 mg/L is an optimal H_2O_2 concentration for the elimination of micro-contaminants in natural water (Miralles-Cuevas et al., 2017). Therefore a similar value was selected for further chemical oxidation experiments (50 mg/L).

For comparison, pCBA degradation rates in real effluent are presented in Figure 6 for the following processes: UV, UV/TiO_2 and UV/H_2O_2. No pCBA degradation were observed in the controls kept under dark conditions because catalyst activation and hydrogen peroxide photolysis requires the presence of UV light. Direct photolysis (only UV) gave a rate of $0.0436 \pm 0.0005\, min^{-1}$ which was only improved by 38% when adding 100 mg/L of catalyst. As aforementioned, catalyst aggregation is a possible reason for such a small enhancement in the real effluent. Replacing the catalyst by 50 mg/L of hydrogen peroxide led to a $0.373 \pm 0.003\, min^{-1}$ degradation rate, one order of magnitude higher than the one obtained by UV/TiO_2. Even 20 mg/L of H_2O_2 performed better than optimized UV/TiO_2. Therefore

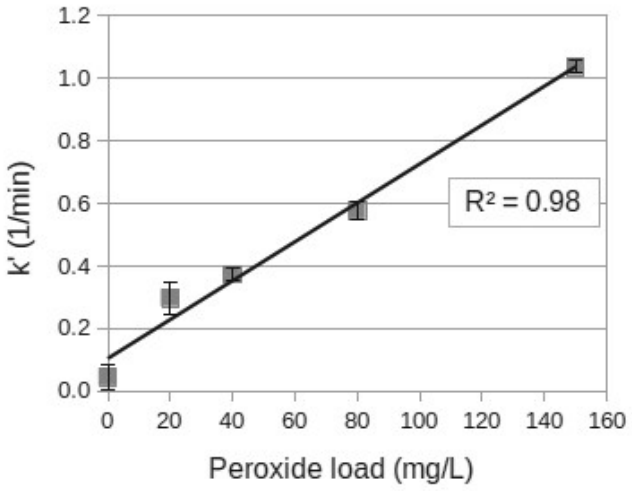


Figure 5: pCBA (5 μM) degradation in function of hydrogen peroxide concentration in secondary effluent

peroxide is an efficient alternative to catalyst when aggregation phenomena limits removal rates of photocatalysis.

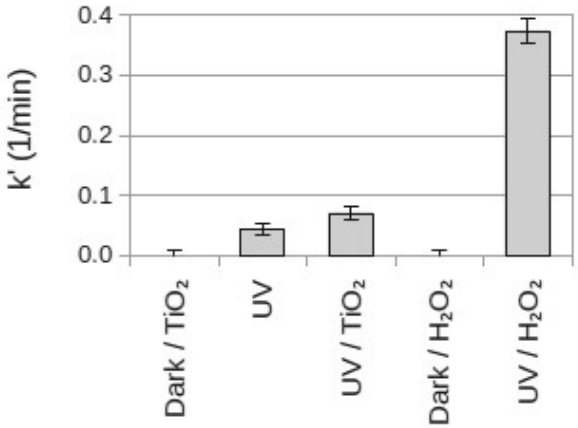


Figure 6: Comparison of UV/TiO_2 (100 mg/L) and UV/H_2O_2 (40 mg/L) for pCBA (5 μM) degradation in secondary effluent

3.3 Degradation of anti-cancer drugs spiked in secondary effluent

UV, UV/TiO_2 and UV/H_2O_2 experiments were conducted on secondary effluent spiked with four anti-cancer drugs at 500 $\mu g/L$ as indicated in section 2.3. After 10 minutes of irradiation, etoposide and paclitaxel could not be detected in the photo-reactor as shown in Figure 7, meaning a removal above 98% and degradation rate higher than $0.46\,min^{-1}$. This is an encouraging result since these lamps are currently installed in water treatment plants for disinfection purposes (EPA, 2006). Degradation of etoposide by photolysis has bee been previously reported corroborating results obtained in this work (de Sousa Filho et al., 2018; Franquet-Griell et al., 2017). However to the best of the authors knowledge, photolysis of pactitaxel has not yet been published in literature. The large molecular structure of paclitaxel and etoposide presented in Figure 1 may explain their low stability toward direct photolysis. The probability to break one of their diverse and numerous chemical bonds is higher for larger molecules. At last, the addition of photocatalyst in the reactor did not either improve nor hinder degradation rates obtained by photolysis of etoposide and paclitaxel. The two other compounds investigated were not removed after 180 minutes of irradiation with and without catalyst or hydrogen peroxide (see Figure 7). The inefficiency of direct photolysis to degrade cyclophosphamide and ifosfamide has been reported in previous works (Franquet-Griell et al., 2017; Lutterbeck et al., 2016). Furthermore Kim et al. ranked cyclophosphamide as the second most resilient compound over 30 pharmaceuticals and personal care products in terms of photo-degradation rates (Kim et al., 2009). The authors linked this stability to the presence of an amine group allowing for resonance stabilization (see molecular structure in Figure 1). This probably explains why our essays to degrade cyclophosphamide and ifosfamide by advanced oxidation processes with TiO_2 (P25) and H_2O_2 in the photoreactor showed negligible removals, equivalent to controls in the dark (see Figure 7).

Other works reported in literature indicate that despite their high stability, successful degradations of cyclophosphamide and ifosfamide were obtained by TiO_2 photocatalysis at similar

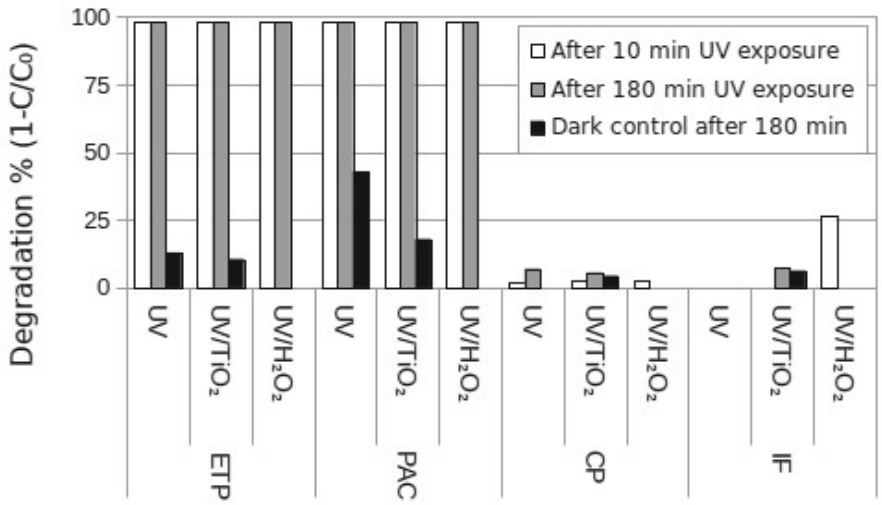


Figure 7: Removal percentages of etoposide (ETP), paclitaxel (PAC), cyclophosphamide (CP) and ifosfamide (IF) spiked at 500 $\mu g/L$ by UV, UV/TiO_2 (100 mg/L) and UV/H_2O_2 (50 mg/L) in secondary wastewater effluent

pollutants levels (Lin and Lin, 2014), water matrix (Fiszka Borzyszkowska et al., 2016) and catalyst concentrations (Lutterbeck et al., 2015) than to ones tested in this work. In addition, Lutterbeck et al. and Zhang et al. observed cyclophosphamide degradation at similar hydrogen peroxide concentrations ($9.8 - 19.6$mM and $1 - 4$ mM) than the one studied in this paper (1.1 mM) (Lutterbeck et al., 2015; Zhang et al., 2017). The higher removal performances achieved by these works may be explained by the use of different reactor configurations as well as lamps irradiance and emission spectrum.

3.4 Degradation of anti-cancer drugs from nanofiltration retentate

In order to reduce the volume to treat by oxidation processes, the secondary effluent spiked with anti-cancer drugs was concentrated by nanofiltration applying a concentration factor of 5. Permeability value were calculated using equation 1 and plotted on figure 8 evidencing that higher permeability were obtained by filtration of laboratory grade water than wastewater

effluent. This is probably related to the presence of ions and natural organic mater in the real matrix (see Table S1 in supp. data). During effluent filtration, no flux decline was observed suggesting that no fouling occurred (see Figure 8). Drug rejections were calculated as explained in section 2.4: etoposite > 98%, paclitaxel > 98%, cyclophosphamide $92 \pm 4\%$ and ifosfamide $85 \pm 7\%$. Similar values were obtained and discussed in our previous work: etoposite $98.7 \pm 0.2\%$, paclitaxel $99 \pm 0.005\%$, cyclophosphamide $90.4 \pm 0.5\%$ and ifosfamide $88.8 \pm 0.2\%$ (Cristóvão et al., 2019). It is worth noting that in the previous publication referenced and this one, same filtration procedures, feed solution and membrane (Desal 5DK) were used.

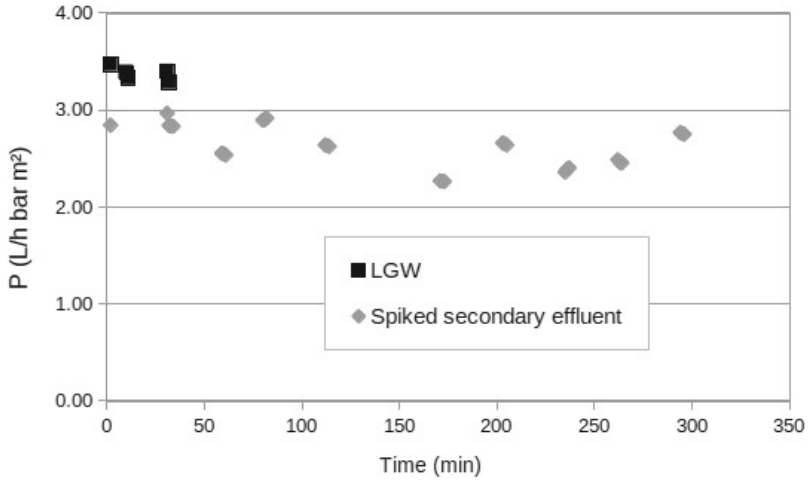


Figure 8: Removal percentages of etoposide (ETP), paclitaxel (PAC), cyclophosphamide (CP) and ifosfamide (IF) spiked at 500 $\mu g/L$ by UV, UV/TiO_2 (100 mg/L) and UV/H_2O_2 (50 mg/L) in secondary wastewater effluent

Drug adsorptions on membrane were also calculated using equation 2: etoposide $42 \pm 7\%$, paclitaxel $32 \pm 22\%$, cyclophosphamide $2 \pm 2\%$ and ifosfamide $5 \pm 3\%$. Knowing that etoposide and paclitaxel are more hydrophobic than the two other drugs (Zhang et al., 2013), their higher tendency to adsorb on membrane surfaces is logical. Higher adsorption of etoposide and paclitaxel than cyclophosphamide and ifosfamide were also observed in our previous nanofiltration work (Cristóvão et al., 2019). Surprisingly, the three oxidation processes removed etoposide and paclitaxel as fast from the NF-concentrate (see Figure 9) as from the initial solution (see

Figure 7). In less than 10 minutes of light exposure these two drugs could not be detected by HPLC-MS/MS. This is an unexpected result as the initial drug concentrations have almost doubled after nanofiltration and the higher salt and natural organic matter content could have hindered light penetration through the media. However as in previous degradation tests presented in Figure 7, cyclophosphamide and ifosfamide did not degrade neither by UV/TiO_2 nor by UV/H_2O_2. Post treatment of nanofiltration retentate by photolysis was effective for the removal of etoposide and paclitaxel. However future experiments should be performed with higher H_2O_2 loads or other treatment processes such as ozonation to optimize the degradation of cyclophosphamide and ifosfamide. In this case, advanced oxidation processes operated on reduced volumes thanks to nanofiltration may stand as an effective barrier against anti-cancer drugs discharge in surface water.

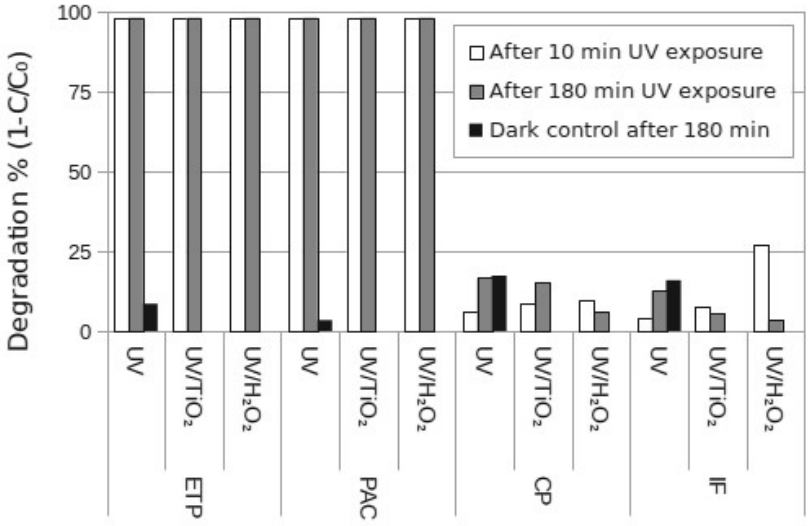


Figure 9: Removal of etoposide (ETP), paclitaxel (PAC), cyclophosphamide (CP) and ifosfamide (IF) from a NF concentrate by UV, UV/TiO2 (100 mg/L) and UV/H2O2 (40 mg/L)

4 Conclusions

Anti-cancer drugs have been detected at ng/L levels in European rivers. The genotoxicity, mutagenicity and fetotoxicity of these emerging contaminants appeals to lower as much as reasonably achievable discharge of cytostatic drugs in surface water. In this work, etoposide, paclitaxel, cyclophosphamide and ifosfamide removals were investigated in secondary effluent spiked at 500 $\mu g/L$. Etoposide and paclitaxel were degraded in less than 10 min by direct photolysis in secondary effluent and similar removal efficiencies were obtained on the concentrated nanofiltration retentate. Therefore installation of medium pressure mercury lamps in wastewater plants may be an effective strategy for removal of etoposide and paclitaxel. Similar results on etoposide were reported in literature, however, to the best of the author's knowledge, the degradation of paclitaxel has not been reported. However the two other drugs investigated, cyclophosphamide and ifosfamide, were not degraded by any of the applied treatments: UV, UV/TiO_2 and UV/H_2O_2 using the exposure times and the concentrations of TiO_2 and H_2O_2 applied. In the case of photocatalysis, catalyst particle aggregation observed only in real matrix was shown to deteriorate formation of OH radicals. This was evidenced thanks to novel results obtained with the hydroxyl molecular probe (pCBA).

The effective drugs rejection ($> 85\%$) obtained by nanofiltration together with the high removal rates of etoposide and paclitaxel from the concentrated secondary wastewater effluent show that combining filtration with oxidation processes stand as an effective barrier against the discharge to surface water of some anti-cancer drugs.

Acknowledgement

The authors thank Joao Ferreira, Pedro Bastos and Joana Quintela for the implementation of the analytical method described in this study. Financial support from the European Commis-

sion through the project ERA-NET Inno Indigo 2014 (Inn-INDIGO/0002/2014) is gratefully acknowledged. iNOVA4Health - UID/Multi/04462/2013, a program financially supported by Fundacao para a Ciencia e Tecnologia/Ministerio da Educacao e Ciencia, through national funds and co-funded by FEDER under the PT2020 Partnership Agreement is gratefully acknowledged. Associate Laboratory for Green Chemistry LAQV - Requimte which is also financed by national funds from FCT/MEC (UID/QUI/50006/2013) and co-financed by the ERDF under the PT2020 Partnership Agreement (POCI-01-0145-FEDER - 007265) is gratefully acknowledged. Authors would also like to acknowledge Aguas do Tejo Atlantico for support. The authors declare no competing financial interest.

Supporting Material

Table S1: Secondary effluent characterization reprinted with authorization form (Cristóvão et al., 2019)

pH at 20°C	7.1
Biochemical oxygen demand (mg/L O_2)	10
Chemical oxygen demand (mg/L O_2)	50
Total suspended solids (mg/L)	8
Nitrogen (mg/L NH_4)	17
Calcium (mg/L)	18.5 ± 1.45
Magnesium (mg/L)	11.9 ± 3.85
Potassium (mg/L)	1606.4 ± 595.12
Sodium (mg/L)	31.6 ± 12.2
Chloride (mg/L)	53.3 ± 5.55
Nitrate (mg/L NO_3)	21.0 ± 0.51
Sulfate (mg/L)	18.2 ± 1.05
Phosphate (mg/L)	nd
Aluminium (mg/L)	nd
Chromium (mg/L)	nd
Iron (mg/L)	0.07 ± 0.003
Zinc (mg/L)	0.01 ± 0.0001

nd: not detectable

References

J P Besse, J F Latour, and J Garric. Anticancer drugs in surface waters. What can we say about the occurrence and environmental significance of cytotoxic, cytostatic and endocrine therapy drugs? *Environment International*, 39(1):73–86, 2012. ISSN 01604120. doi: 10.1016/j.envint.2011.10.002. URL http://dx.doi.org/10.1016/j.envint.2011.10.002.

A Boedec, C Albasi, J Grollemund, C Dehan, and H Roux-De-Balmann. Traitement par nanofiltration d'effluents aqueux polysiloxaniques : influence des matrices salines. 30(1): 20–27, 2017.

V Booker, C Halsall, N Llewellyn, A Johnson, and R Williams. Prioritising anticancer drugs for environmental monitoring and risk assessment purposes. *Science of The Total Environment*, 473-474:159–170, mar 2014. ISSN 00489697. doi: 10.1016/j.scitotenv.2013.11.145. URL http://dx.doi.org/10.1016/j.scitotenv.2013.11.145.

L Brunet, D Lyon, E Hotze, P Alvarez, and M Wiesner. Comparative photoactivity and antibacterial properties of C60 fullerenes and titanium dioxide nanoparticles. *Environmental science & technology*, 43:4355–4366, 2009. doi: 10.1016/S0169-7161(06)26025-5.

B Cristóvão, J Torrejais, R Janssens, P Luis, B. Van der Bruggen, K K Dubey, M K Mandal, M R Bronze, J. G. Crespo, and V. J. Pereira. Membrane filtration to treat anticancer drugs in hospital and domestic wastewater effluents. *Separation and Purification Technology*, 224: 273–280, 2019. doi: 10.1016/j.seppur.2019.05.016.

I A de Sousa Filho, T M Lobo, C K Grisolia, I T Weber, and M E Osugi. Toxicological study of the degradation products of antineoplastic agent etoposide in commercial formulation treated by heterogeneous photocatalysis using SrSnO3. *Environmental Science and Pollution Research*, pages 1–10, 2018. ISSN 16147499. doi: 10.1007/s11356-018-1524-2.

A Eitel, M Scherrer, and K Kummerer. Handling cytostatic drugs : A practical guide. 1999.

M.S. Elovitz and U. Von Gunten. Hydroxyl radical/ozone ratios during ozonation processes. I. The R(ct) concept. *Ozone: Science and Engineering*, 21(3):239–260, 1999. doi: 10.1080/01919519908547239.

EPA. Ultraviolet Disinfection Guidance Manual for the Final Long Term 2 Enhanced Surface Water Treatment Rule, EPA 815-R-06-007. *US Environmental protection Agency*, 2006.

C Escudero-Onate, L Ferrando-Climent, S Rodriguez-Mozaz, and L Santos. Occurrence and risks of contrast agents, cytosatics and antibiotics in hospital effluents. In *Verlicchi P. (eds) Hospital wastewater*. Hdb Env Chem, Springer, 2017. ISBN 978-3-319-62177-7. doi: 10.1007/698_2017_12.

A Fiszka Borzyszkowska, A Pieczyńska, A Ofiarska, K Nikiforow, P Stepnowski, and E M Siedlecka. Bi-B-TiO2-based photocatalytic decomposition of cytostatic drugs under simulated sunlight treatments. *Separation and Purification Technology*, 169:113–120, 2016. ISSN 18733794. doi: 10.1016/j.seppur.2016.06.012.

H Franquet-Griell, C Gómez-Canela, F Ventura, and S Lacorte. Predicting concentrations of cytostatic drugs in sewage effluents and surface waters of Catalonia (NE Spain). *Environmental Research*, 138:161–172, 2015. ISSN 10960953. doi: 10.1016/j.envres.2015.02.015. URL http://dx.doi.org/10.1016/j.envres.2015.02.015.

H Franquet-Griell, A Medina, C Sans, and S Lacorte. Biological and photochemical degradation of cytostatic drugs under laboratory conditions. *Journal of Hazardous Materials*, 323:319–328, 2017. ISSN 18733336. doi: 10.1016/j.jhazmat.2016.06.057. URL http://dx.doi.org/10.1016/j.jhazmat.2016.06.057.

R Janssens, B Cristóvão, M R Bronze, J G Crespo, V J Pereira, and P Luis. Photocatalysis using UV-A and UV-C light sources for advanced oxidation of anti-cancer drugs spiked in laboratory grade water and synthetic urine (Unpublished work: Paper submitted on the 11/05/2019, available as supplementary material). *Chemosphere*.

R Janssens, M K Mandal, K K Dubey, and P Luis. Slurry photocatalytic membrane reactor technology for removal of pharmaceutical compounds from wastewater: Towards cytostatic drug elimination. *Science of the Total Environment*, 599-600:612–626, 2017. ISSN 0048-9697. doi: 10.1016/j.scitotenv.2017.03.253. URL http://dx.doi.org/10.1016/j.scitotenv.2017.03.253.

S H Joo and B Tansel. Novel technologies for reverse osmosis concentrate treatment: A review. *Journal of Environmental Management*, 150:322–335, 2015. ISSN 10958630. doi: 10.1016/j.jenvman.2014.10.027. URL http://dx.doi.org/10.1016/j.jenvman.2014.10.027.

I Kim, N Yamashita, and H Tanaka. Photodegradation of pharmaceuticals and personal care products during UV and UV/H2O2 treatments. *Chemosphere*, 77(4):518–525, 2009. ISSN 00456535. doi: 10.1016/j.chemosphere.2009.07.041.

K Kimura, T Iwase, S Kita, and Y Watanabe. Influence of residual organic macromolecules produced in biological wastewater treatment processes on removal of pharmaceuticals by NF/RO membranes. *Water Research*, 43(15):3751–3758, aug 2009. ISSN 0043-1354. doi: 10.1016/J.WATRES.2009.05.042. URL https://www.sciencedirect.com/science/article/pii/S0043135409003674.

I Koltsakidou, M Antonopoulou, E Evgenidou, I Konstantinou, and D A Lambropoulou. Cytarabine degradation by simulated solar assisted photocatalysis using TiO2. *Chemical Engineering Journal*, 316:823–831, 2017. ISSN 1385-8947. doi: 10.1016/j.cej.2017.01.132. URL http://dx.doi.org/10.1016/j.cej.2017.01.132.

K Kummerer, A Al-ahmad, B Bertram, and M Wieûler. Biodegradability of antineoplastic compounds in screening tests : influence of glucosidation and of stereochemistry. *Chemosphere*, 40:767–773, 2000.

W Lai, H Lin, and A Y-C Lin. TiO2 photocatalytic degradation and transformation of oxazaphosphorine drugs in an aqueous environment. *Journal of Hazardous Materials*, 287:133–141, 2015. ISSN 0304-3894. doi: 10.1016/j.jhazmat.2015.01.045. URL http://dx.doi.org/10.1016/j.jhazmat.2015.01.045.

K Lenz, G Koellensperger, S Hann, N Weissenbacher, S N Mahnik, and M Fuerhacker. Fate of cancerostatic platinum compounds in biological wastewater treatment of hospital effluents. *Chemosphere*, 69(11):1765–1774, 2007. doi: 10.1016/j.chemosphere.2007.05.062.

H H H Lin and A Y-C Lin. Photocatalytic oxidation of 5-fluorouracil and cyclophosphamide via UV/TiO2 in an aqueous environment. *Water Research*, 48(1):559–568, 2014. ISSN 00431354. doi: 10.1016/j.watres.2013.10.011. URL `http://dx.doi.org/10.1016/j.watres.2013.10.011`.

C Lutterbeck, M Wilde, E Baginska, C Leder, E MacHado, and K Kummerer. Degradation of cyclophosphamide and 5-fluorouracil by UV and simulated sunlight treatments: Assessment of the enhancement of the biodegradability and toxicity. *Environmental Pollution*, 208:467–476, 2016. ISSN 18736424. doi: 10.1016/j.envpol.2015.10.016.

C A Lutterbeck, E L Machado, and K Kümmerer. Photodegradation of the antineoplastic cyclophosphamide : A comparative study of the efficiencies of UV/H2O2 , UV/Fe2+/H2O2 and UV/TiO2 processes. *Chemosphere*, 120:538–546, 2015. ISSN 00456535. doi: 10.1016/j.chemosphere.2014.08.076.

S N Mahnik. Fate of 5-fluorouracil , doxorubicin , epirubicin , and daunorubicin in hospital wastewater and their elimination by activated sludge and treatment in a membrane-bioreactor system. *Chemosphere*, 66:30–37, 2007. doi: 10.1016/j.chemosphere.2006.05.051.

S Miralles-Cuevas, D Darowna, A Wanag, S Mozia, S Malato, and I Oller. Comparison of UV/H2O2, UV/S2O82, solar/Fe(II)/H2O2 and solar/Fe(II)/S2O82 at pilot plant scale for the elimination of micro-contaminants in natural water: An economic assessment. *Chemical Engineering Journal*, 310:514–524, 2017. ISSN 13858947. doi: 10.1016/j.cej.2016.06.121. URL `http://dx.doi.org/10.1016/j.cej.2016.06.121`.

R Molinari, A Caruso, P Argurio, and T Poerio. Degradation of the drugs Gemfibrozil and Tamoxifen in pressurized and de-pressurized membrane photoreactors using suspended polycrystalline TiO2 as catalyst. *Journal of Membrane Science*, 319(1-2):54–63, 2008. ISSN 03767388. doi: 10.1016/j.memsci.2008.03.033.

N Negreira, M López de Alda, and D Barceló. Study of the stability of 26 cytostatic drugs and metabolites in wastewater under different conditions. *Science of the Total Environment*, 482-483(1):389–398, 2014. ISSN 18791026. doi: 10.1016/j.scitotenv.2014.02.131. URL `http://dx.doi.org/10.1016/j.scitotenv.2014.02.131`.

Matjaž Novak, Bojana Žegura, Barbara Modic, Ester Heath, and Metka Filipič. Cytotoxicity and genotoxicity of anticancer drug residues and their mixtures in experimental model with zebrafish liver cells. *Science of the Total Environment*, 601-602:293–300, 2017. ISSN 18791026. doi: 10.1016/j.scitotenv.2017.05.115.

A Ofiarska, A Pieczyńska, A F Borzyszkowska, P Stepnowski, and E M Siedlecka. Pt-TiO2-assisted photocatalytic degradation of the cytostatic drugs ifosfamide and cyclophosphamide under artificial sunlight. *Chemical Engineering Journal*, 285:417–427, 2016. ISSN 13858947. doi: 10.1016/j.cej.2015.09.109.

S Ottofuelling, F Von Der Kammer, and T Hofmann. Commercial titanium dioxide nanoparticles in both natural and synthetic water: Comprehensive multidimensional testing and prediction of aggregation behavior. *Environmental Science and Technology*, 45(23):10045–10052, 2011. ISSN 0013936X. doi: 10.1021/es2023225.

V J Pereira, H S Weinberg, K G Linden, and P C Singer. UV degradation kinetics and modeling of pharmaceutical compounds in laboratory grade and surface water via direct and indirect photolysis at 254 nm. *Environmental Science and Technology*, 41(5):1682–1688, 2007. ISSN 0013936X. doi: 10.1021/es061491b.

J. Radjenović, M. Petrović, F. Ventura, D. Barceló, M Petrovic, F. Ventura, and D Barcelo. Rejection of pharmaceuticals in nanofiltration and reverse osmosis membrane drinking water treatment. *Water Research*, 42(14):3601–3610, aug 2008. ISSN 0043-1354. doi: 10.1016/J.WATRES.2008.05.020. URL https://www.sciencedirect.com/science/article/pii/S0043135408002248.

E Rosenfeldt, K G Linden, S Canonica, and U Von Gunten. Comparison of the efficiency of OH radical formation during ozonation and the advanced oxidation processes O3/H2O2 and UV/H2O2. *Water Research*, 40:3695–3704, 2006. doi: 10.1016/j.watres.2006.09.008.

H Sanderson, R Brain, D Johnson, C J Wilson, and K R Solomon. Toxicity classification and evaluation of four pharmaceuticals classes : antibiotics , antineoplastics , cardiovascular , and sex hormones. *Toxicology*, 203:27–40, 2004.

M Taheran, S K Brar, M Verma, R Y Surampalli, T C Zhang, and J R Valero. Membrane processes for removal of pharmaceutically active compounds (PhACs) from water and wastewaters. *Science of The Total Environment*, 547:60–77, mar 2016. ISSN 0048-9697. doi: 10.1016/J.SCITOTENV.2015.12.139. URL https://www.sciencedirect.com/science/article/pii/S0048969715312948.

A P Toolaram, K Kümmerer, and M Schneider. Environmental risk assessment of anti-cancer drugs and their transformation products: A focus on their genotoxicity characterization-state of knowledge and short comings. *Mutation Research - Reviews in Mutation Research*, 760: 18–35, 2014. ISSN 13882139. doi: 10.1016/j.mrrev.2014.02.001.

E Topuz, J Traber, L Sigg, and I Talinli. Agglomeration of Ag and TiO2 nanoparticles in surface and wastewater: Role of calcium ions and of organic carbon fractions. *Environmental Pollution*, 204:313–323, sep 2015. ISSN 0269-7491. doi: 10.1016/J.ENVPOL.2015.05.034. URL https://www.sciencedirect.com/science/article/pii/S0269749115002687.

M Umar and H Aziz. Photocatalytic Degradation of Organic Pollutants in Water. chapter 8. Intech, 2012.

J Zhang, V W C Chang, A Giannis, and J-Y Y Wang. Removal of cytostatic drugs from aquatic environment: a review. *Science of the Total Environment*, 445-446:281–98, 2013. ISSN 00489697. doi: 10.1016/j.scitotenv.2012.12.061. URL http://www.ncbi.nlm.nih.gov/pubmed/23337605.

Y Zhang, Y Xiao, J Zhang, V Chang, and T-T Lim. Degradation of cyclophosphamide and

5-fluorouracil in water using UV and UV/H2O2: Kinetics investigation, pathways and energetic analysis. *Journal of Environmental Chemical Engineering*, 5(1):1133–1139, 2017. ISSN 22133437. doi: 10.1016/j.jece.2017.01.013. URL http://linkinghub.elsevier.com/retrieve/pii/S2213343717300143.

Y.-Y. Zhao, F.-X. Kong, Z. Wang, H.-W. Yang, X.-M. Wang, Y.F. Xie, and T.D. Waite. Role of membrane and compound properties in affecting the rejection of pharmaceuticals by different RO/NF membranes. *Frontiers of Environmental Science and Engineering*, 11(6), 2017. doi: 10.1007/s11783-017-0975-x.