Degradation of cefuroxime in aqueous TiO$_2$ suspensions under simulated solar radiation

KOYTANTOY V[1]., TYROVOLA K[1]., DROSOU C[1]. and XEKOUKOULOTAKIS N.P.[1,*].

[1]School of Environmental Engineering, Technical University of Crete, University Campus, GR-73100 Chania, Greece

*corresponding author: Xekoukoulotakis, N.P
e-mail: nikosxek@gmail.com

Abstract The photocatalytic degradation of the antibiotic cefuroxime was studied under simulated solar radiation in aqueous suspensions of a commercially available TiO$_2$ catalyst (Aeroxide-P25). The effect of various parameters, as the initial concentration of cefuroxime, TiO$_2$ concentration and pH value, was investigated. In addition, the photocatalytic degradation of cefuroxime in complex water matrices (waste water and synthetic fresh and hydrolyzed urine), was studied. Moreover, in order to investigate the mechanism of photocatalytic oxidation of cefuroxime, the effect of addition of isopropanol, a strong inhibitor of the free hydroxyl radicals, HO•, was studied.

Keywords: TiO$_2$ solar photocatalysis, antibiotic, cefuroxime.

1. Introduction

During the last years, the increasing presence of organic and inorganic pollutants in the aquatic environment requires proper management, treatment and re-use of water and wastewater, to protect the environment and public health. However, the conventional treatment methods have been found to be ineffective to remove various synthetic organic compounds, such as pharmaceuticals. Consequently, it is important to apply advanced treatment methods capable to remove various organic pollutants of emerging interest from water and wastewater.

The present study was focused on the TiO$_2$ photocatalytic degradation of cefuroxime, in aqueous solutions under solar simulated irradiation. Cefuroxime belongs to the group of semi-synthetic cephalosporins, a widely used class of b-lactam antibiotics. In recent years, several antibiotics which belong to the class of cephalosporins, including cefuroxime, have been detected in the aquatic environment at various concentrations.

2. Materials and Methods

2.1. Chemicals and reagents

The experiments were conducted using cefuroxime sodium salt (C$_{16}$H$_{15}$N$_4$NaO$_8$S) purchased from Sigma-Aldrich. TiO$_2$ photocatalyst (P25 Aeroxide) purchased from Evonik. Phosphate salts, such as Na$_2$HPO$_4$·H$_2$O and KH$_2$PO$_4$ (both obtained from Merck) were employed for the preparation of aqueous buffer solutions. Sodium phosphate monobasic monohydrate (NaH$_2$PO$_4$·H$_2$O) was obtained from Sigma-Aldrich and acetonitrile was purchased from Merck and both were used as solvents for high performance liquid chromatography (HPLC). All aqueous solutions were prepared with ultrapure water from the purification system Simplicity UV supplied by Millipore.

Table 1. Chemical composition of synthetic fresh and hydrolyzed urine (Zhang et al., 2015)

Species	mol·L^{-1}	g·L^{-1}	mol·L^{-1}	g·L^{-1}
	SFU		SHU	
Urea	0.25	15.015	0	0
NaCl	0.044	2.571	0,06	3,506
Na$_2$SO$_4$	0.015	2.131	0,015	2,131
KCl	0.04	2.982	0,04	2,982
NH$_4$OH (conc)	0	0	0,25	17,200
MgCl$_2$·6H$_2$O	0.004	0.813	0	0
NaH$_2$PO$_4$·H$_2$O	0.02	2.760	0,0136	1,877
CaCl$_2$·2H$_2$O	0.004	0.588	0	0
NH$_4$HCO$_3$	0	0	0,25	19,765
Na$_3$Citrate·2H$_2$O	0.0027	0.794	0	0
pH	6		9	

In order to investigate the effect of water matrices, the experiments were performed in three types of water: the secondary treated wastewater effluent (WW), synthetic fresh urine (SFU) and hydrolyzed urine (SHU). The chemical composition of SFU, SHU and WW is presented at Tables 1 and 2.

Table 2. Chemical composition of the secondary treated wastewater (WW) effluent

Physicochemical properties of waste water (WW)	
pH	8,1
Conductivity, μS/cm	832
Alkalinity (mg/L of CaCO₃)	77,5
COD, in mg/L	11
Non-purgeable organic carbon (NPOC), mg/L	6,2
$[Ca^{2+}]$, mg/L	43,12
$[Mg^{2+}]$, mg/L	17,46
$[Na^+]$, mg/L	119,86
$[K^+]$, mg/L	18,11
$[NH_4^+]$, mg/L	2,00
$[HCO_3^-]$, mg/L	207,98
$[Cl^-]$, mg/L	146,48
$[SO_4^{2-}]$, mg/L	90,15
$[NO_3^-]$, mg/L	5,62
$[PO_4^{3-}]$, mg/L	0,06

Stock solution of cefuroxime was prepared by dissolving the appropriate amount of antibiotic in each matrix. The exact concentration of the resulting aqueous stock solutions was measured by HPLC.

### 2.2. TiO₂ solar photocatalysis experiments

Photocatalytic experiments were performed in an Ace Glass laboratory reactor (Vineland NJ, USA 1936). The reactor consists of a cylindrical vessel into which the cefuroxime solution was introduced. To illuminate the solution, Newport's solar radiation simulator was equipped with a 150W Xe lamp, using suitable filters to simulate solar irradiation. In a typical photocatalysis run, 300 mL of the aqueous solution containing the desired concentration of cefuroxime was loaded in the reaction vessel and mixed with appropriate quantity of TiO₂ catalyst (P 25 Aeroxide), about 30 minutes in dark, to achieve an adsorption-desorption balance on the surface of the photocatalyst. During the 30 minutes, the cylindrical vessel was covered by aluminum foil so that adsorption was not affected by external irradiation. The first sample was taken immediately before the catalyst is placed in the solution. Two extra samples were also taken, one at 15 minutes of stirring and the other at the end of the stirring in the dark, i.e. after 30 minutes. After the end of the 30 minutes of adsorption in the dark, the aluminum foil was removed and the lamp was turned on. Samples were taken at regular intervals and then were centrifuged for 15 min at 13200 rpm to separate the catalyst from the solution. In continue, the solution was carefully collected and was introduced into a suitable 2 mL vial which is placed in the HPLC system for measurement. The pH value was measuring at the beginning and end of the experiment. The experiments were carried out at ambient conditions (i.e. at 25 ° C).

2.3 Analytical Methods

Cefuroxime concentration was determined at 273 nm, using HPLC (Alliance 2695 Waters with a Diode Array (PDA Detector)) and a Supelco 5.0 um C18 column by Sigma Aldrich (250 mm×4.6 mm). The mobile phase was a mixture of phosphate buffer 20mM and acetonitrile using a gradient program with a flow rate of 1 mL·min⁻¹. The gradient started with 90% phosphate buffer and this composition was held constant for the 1st minute. After the 1st minute the composition of phosphate buffer proceeded to 80% till the 8th minute and then returned to the initial conditions for a further 3 minutes. The temperature of the column compartment was 30°C, and the injected volume for HPLC analysis was 100 μL.

3. Results & Discussion

In an initial set of photolysis experiments, the direct photolysis, under solar simulated irradiation, of cefuroxime was studied at an initial concentration of 3.36 μmol L⁻¹ and at solution pH 7. The results are shown in Figures 1.
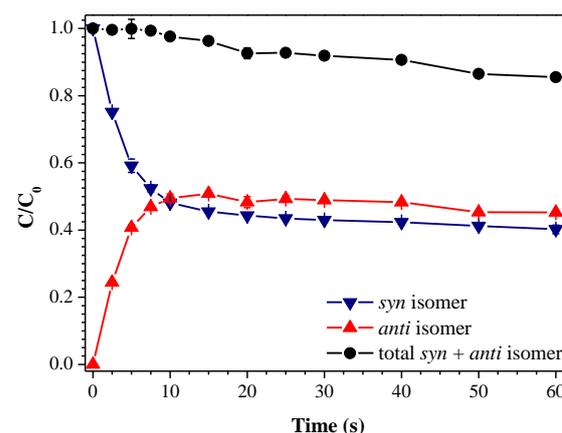
**Figure 1.** Direct photolysis of cefuroxime, under solar silulated irradiation. Experimental conditions: [cefuroxime]₀ = 3,36 μmol·L⁻¹, pH=7.

Cephalosporin compounds, including cefuroxime, possessing an alkox-imino function , $R_2O−N=C$ in the 7-sidechain of Δ^3-cephem ring excibit in two syn (or Z) and anti (or E) isomers. The commercially available cephalosporins, are found in the form of the syn(Z)-isomer since the anti-isomer exhibits much less activity as an antibiotic (Lerner et al., 1988). It is also known from the literature that cephalosporins can be photochemically isomerized from syn(Z)-isomer to anti(E)-isomer, as can been seen in Figure 2. As can be seen in Figure1, the concentration of the syn(Z)-isomer of cefuroxime (i.e., the commercially available form of the substance) decreased relatively rapidly (almost 45%) during the first 15 minutes of irradiation, followed by a relatively slower rate of decrease in

concentration. At the same time, cefuroxime anti(E)-isomer concentration was increased during the first 15 minutes of irradiation (about 51% of the initial concentration of cefuroxime), followed by a relatively small decrease in its concentration and after 60 minutes of irradiation its concentration was approximately 45% of the initial concentration of cefuroxime. In addition, the total concentration of the two isomers of cefuroxime shown a relatively slow reduction and after 60 minutes of irradiation, the reduction of the total concentration of the two isomers was about 15%. The above results can be explained considering that during irradiation of a cefuroxime solution, two photochemical transformation take place: (a) the isomerization of syn(Z)-isomer to anti(E)-isomer and the photochemical opening of b-lactamic ring and Δ^3-cephem ring (Figure 2). In addition, it is concluded that the contribution of these photochemical reactions to the total conversion observed during photocatalytic degradation of cefuroxime is practically negligible.
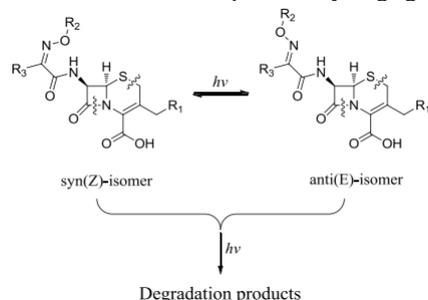
**Figure 2.** Photochemical degradation of cephalosporins

In addition, the effect of TiO_2 concentration was studied at an initial concentration of cefuroxime 3.36 $\mu mol \cdot L^{-1}$ and solution pH 7, while TiO_2 concentration was ranging from 50 $mg \cdot L^{-1}$ to 750 $mg \cdot L^{-1}$.
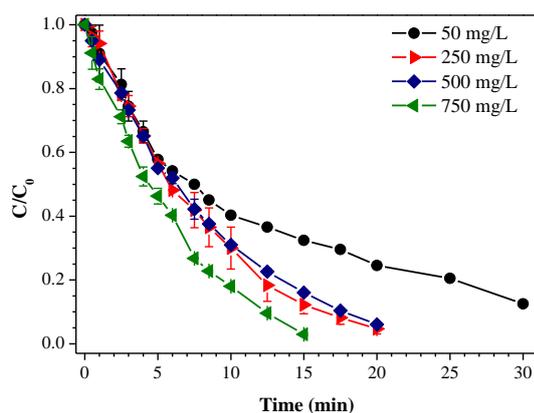
Figure 3. Effect of TiO_2 concentration on cefuroxime degradation. [cefuroxime]= 3.36 $\mu mol \cdot L^{-1}$ (1.5 $mg \cdot L^{-1}$), pH=7.

Moreover, the initial rate of reaction was calculated by fitting a second order polynomial to the experimental results and calculating the polynomial derivative at time zero. The results are shown in Figures 3 and 4.

As can been seen, the initial rate of cefuroxime degradation was increased with increasing of catalyst concentration to 500 $mg \cdot L^{-1}$. Further addition of catalyst doesn't affect to photocatalytic performance.
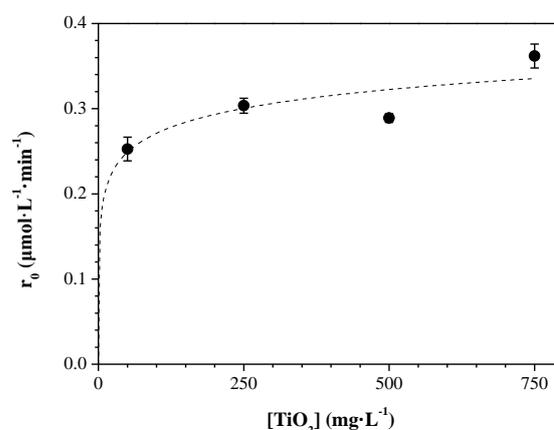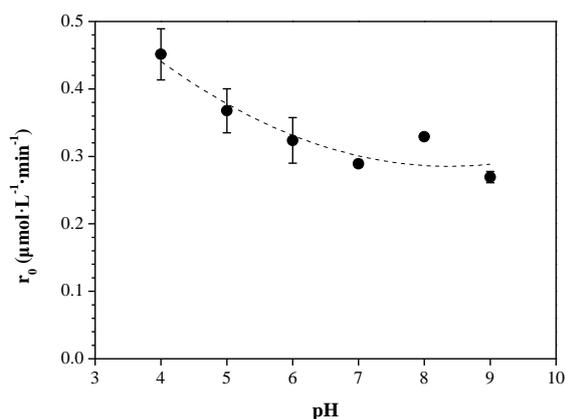

Figure 4. Initial rate, r_o, of cefuroxime photocatalysis versus TiO_2 concentration

The pH effect was studied at cefuroxime solution of initial concentration 3.36 $\mu mol \cdot L^{-1}$ and TiO_2 concentration 500 $mg \cdot L^{-1}$. The pH value was ranging from 4 to 9. The results are shown in Figures 5 and 6.
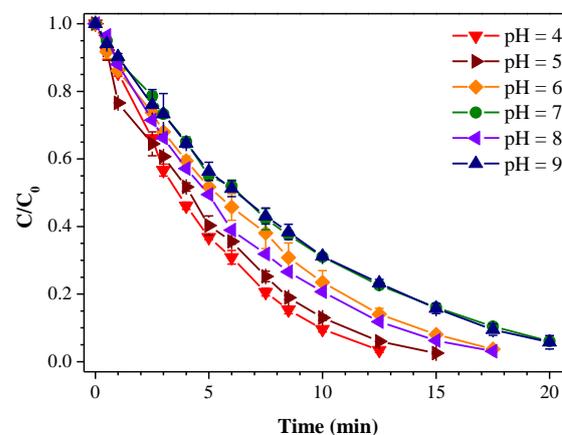
**Figure 5.** Effect of pH on cefuroxine degradation. [cefuroxime]$_0$ =3.36 $\mu mol \cdot L^{-1}$ (1.5 $mg \cdot L^{-1}$), [TiO_2]=500 $mg \cdot L^{-1}$.

A can been seen in Figure 5 the photocatalytic degradation of cefuroxime is favored at acidic condition (i.e. pH<6) (Figure 6).

**Figure 6**. Initial rate, r_o, of cefuroxime photocatalysis versus pH value

Moreover, the effect of initial concentration of cefuroxime was studied with its initial concentration ranging from 0.505 $\mu mol \cdot L^{-1}$ to 8.592 $\mu mol \cdot L^{-1}$, at solution pH 7 and TiO_2 concentration 500 $mg \cdot L^{-1}$. The results are shown in Figures 7 (a) and (b). Moreover, the initial rate of reaction was calculated by fitting a second order polynomial to the experimental results and calculating the polynomial derivative at time zero (data not shown).
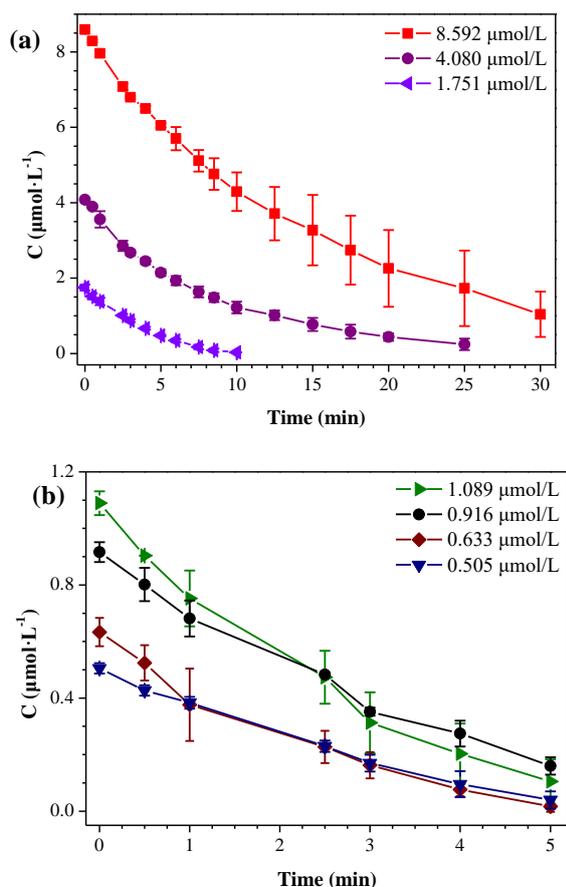
Figure 7 a & b. Effect of initial concentration of cefuroxime. Experimental conditions: $[TiO_2]$ = 500 $mg \cdot L^{-1}$, pH=7

It was found that the initial rate of cefuroxime photocatalytic degradation was increasing from 0.127 $\mu mol \cdot L^{-1} \cdot min^{-1}$ to 0.589 $\mu mol \cdot L^{-1} \cdot min^{-1}$ by increasing the initial concentration of cefuroxime, from 0.505 $\mu mol \cdot L^{-1}$ to 8.592 $\mu mol \cdot L^{-1}$.

The effect of complex water matrices, as wastewater (WW), synthetic fresh urine (SFU) and synthetic hydrolyzed urine (SHU), was studied. The results are shown in Figure 8. As the pH value of WW, SFU and SHU was 8.1, 6 and 9, respectively, for comparison purposes, the results of the experiments carried out in UPW, at corresponding pH values, are also presented.
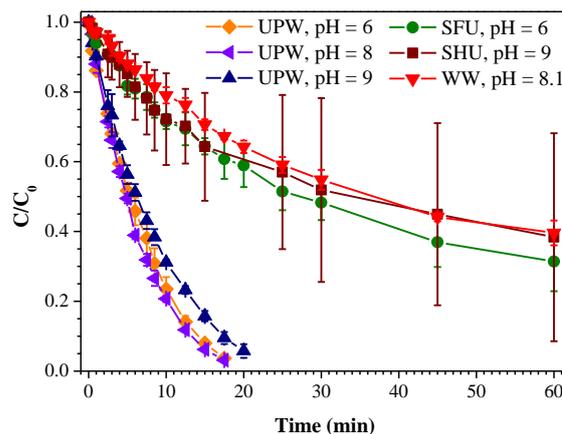

Figure 8. Effect of water matrix on cefuroxime photocatalytic degradation. Experimental conditions: $[cefuroxime]_0$ = 3.36 $\mu mol \cdot L^{-1}$ (1.50 $mg \cdot L^{-1}$), $[TiO_2]$=500 $mg \cdot L^{-1}$.

As can been seen in Figure 8, the complex matrices have a negative effect on the photocatalytic performace compared to UPW. The above results can be explained by the presence of dissolved organic carbon and various inorganic ions, including the bicarbonate ions, in the above aqueous matrices. The presence of these dissolved components act antagonistic to the hydroxyl radicals and other oxidizing chemical species formed during the irradiation of the TiO_2, resulting in a reduction in the performance of photocatalytic oxidation of cefuroxime.

In order to investigate the mechanism of photocatalytic oxidation of cefuroxime, the effect of the addition of isopropanol (IPA) to the reaction mixture was studied. Isopropanol is known to act as an inhibitor of free hydroxyl radicals, HO· (Palominos et al., 2008, van Doorslaer et al., 2012, Antonopoulou and Konstantinou, 2014). The second-order reaction rate constant between the isopropanol and the free hydroxyl radicals equals 1.9 x 10^9 L·mol $^{-1}$ ·s $^{-1}$ and is one of the highest value that exist. The results are shown in Figures 9 and 10. The addition of isopropanol to the reaction mixture caused a significant reduction in the initial reaction rate. In particular, the addition of isopropanol at concentrations ranging from 0.065 $mmol \cdot L^{-1}$ to 1.31 $mmol \cdot L^{-1}$, caused a 60%-90% reduction of initial rate of the reaction. Further increase

of isopropanol concentration did not affect the initial rate. These results indicate that free hydroxyl radicals participate to photocatalytic oxidation of cefuroxime for about 90%, while the remaining oxidizing species, such as the holes, h^+, at valence band of the catalyst, participate only for about 10%.
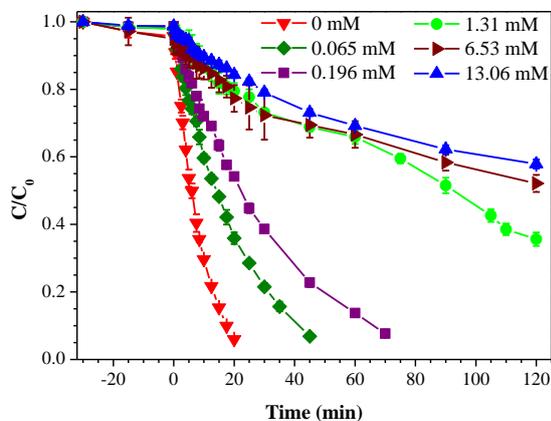
Figure 9. Effect of isopropanol concentration during cefuroxime photocatalysis. [cefuroxime]$_0$ = 3.36 μmol·L^{-1} (1.50 mg·L^{-1}), [TiO$_2$]=500 mg·L^{-1}, pH=7
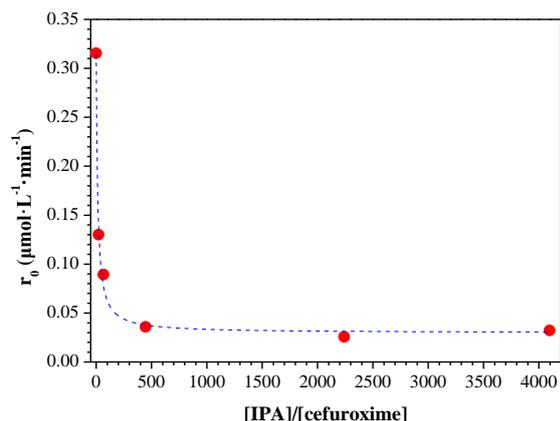
Figure 10. Initial rate, r$_o$, of cefuroxime photocatalysis versus isopropanol-cefuroxime ratio

4. Conclusions

According to the above results, TiO$_2$ solar photocatalysis is a very efficient method for the degradation of the antibiotic cefuroxime. The effect of various parameters as pH value, TiO$_2$ concentration, initial antibiotic concentration and water matrices, was studied. Specifically, it was found that catalyst concentration affects on the performance of the photocatalytic process, with optimum catalyst

concentration to be 500 mg·L^{-1} TiO$_2$. Also, the initial rate of the reaction was slightly increased in acidic pH values, while in neutral or alkaline pH values it remained practically unaffected. Moreover, the initial rate of reaction was increased as the initial concentration of the antibiotic increased. Also, the initial rate of reaction and the overall performance of the photocatalytic process was significantly affected by the nature of the aqueous matrix. Finally, it was found that free hydroxyl radicals participate to photocatalytic oxidation of cefuroxime about 90%, while the remaining oxidizing species, such as the holes, h^+, at valence band of the catalyst, participate only about 10%.

References

Antonopoulou M., Konstantinou I., (2014), Photocatalytic treatment of metribuzin herbicide over TiO$_2$ aqueous suspensions: Removal efficiency, identification of transformation products, reaction pathways and ecotoxicity evaluation. Journal of Photochemistry and Photobiology A: Chemistry 294, 110–120

Lerner D.A., Bonnefond G., Fabre H., Mandrou B., De Simeon Buochberg M., (1988), Photodegradation paths of cefotaxime. Journal of Pharmaceutical Sciences 77 (8) 699–703.

Palominos R., Freer J., Mondaca M.A., Mansilla H.D. (2008), Evidence for hole participation during the photocatalytic oxidation of the antibiotic flumequine. Journal of Photochemistry and Photobiology A: Chemistry 193 (2–3) 139–145

van Doorslaer X., Heynderickx P.M., Demeestere K., Debevere K., Van Langenhove H., Dewulf J. (2012), TiO$_2$ mediated heterogeneous photocatalytic degradation of moxifloxacin: Operational variables and scavenger study. Applied Catalysis B: Environmental 111– 112, 150– 156

Zhang R., Sun P., Boyer T.H., Zhao L., Huang C.H., 2015. Degradation of pharmaceuticals and metabolite in synthetic human urine by UV, UV/H$_2$O$_2$, and UV/PDS. Environmental Science and Technology 49 (5), 3056–3066