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Towards the Removal of Antibiotics Detected in Wastewaters in the POCTEFA Territory: Occurrence and TiO₂ Photocatalytic Pilot-Scale Plant Performance

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Abstract: This research aims to assess the presence of four antibiotic compounds detected in the influent and effluent of wastewater treatment plants (WWTPs) in the POCTEFA territory (north of Spain and south of France) during the period of 2018–2019, and to relate the removal of antibiotic compounds with the processes used in the WWTPs. The performance of a photocatalytic TiO₂/UV-VIS pilot-scale plant was then evaluated for the degradation of selected antibiotics previously detected in urban treated effluent. The main results reflect that azithromycin had the highest mass loadings (11.3 g/day per 1000 inhabitants) in the influent of one of the selected WWTPs. The results also show considerable differences in the extent of antibiotics removal in WWTPs ranging from 100% for sulfadiazine to practically 0% for trimethoprim. Finally, the photocatalytic TiO₂/UV-VIS pilot-scale plant achieved the removal of the four antibiotics after 240 min of treatment from 78%–80% for trimethoprim and enrofloxacin, up to 100% for amoxicillin, sulfadiazine and azithromycin. The catalyst recovery via mechanical coagulation–flocculation–decantation was almost total. The Ti concentration in the effluent of the TiO₂/UV-VIS pilot-scale plant was lower than 0.1% (w/w), and its release into the environment was subsequently minimized.

Keywords: antibiotics; wastewater; removal efficiency; photocatalysis; slurry reactor

Highlights:

- Antibiotics mass loadings range from 11,332 mg/day·1000 inhabitants to undetectable levels.
- Sulfadiazine, amoxicillin and azithromycin can be removed from wastewaters, while 80% of trimethoprim and enrofloxacin removal can be achieved after the photocatalytic treatment.
- The facilities provided with trickling filters proved to be more effective in removing antibiotics from wastewaters.

1. Introduction

The problem of the presence of pharmaceutical compounds in wastewater has recently become a matter for concern, not only in terms of human health, but also for the preservation of the environment [1].

Antibiotics are an important group of medicines suitable for the treatment of human infections and in veterinary medicine. Many of them are not completely metabolized by the body so between 30%–90% are excreted and, as a result, they end up in wastewater [2,3]. The main difference with other organic pollutants is that antibiotics represent a potential risk if they are released into the environment because they have a direct biological action on microorganisms, generating antimicrobial-resistant bacteria (ARB). As suggested by other authors [4], ARB of animal origin can also be transmitted to humans.

Several studies have pointed out that conventional wastewater treatment plants (WWTPs) are not designed to remove pharmaceuticals, metabolites or drugs [3–5]. Besides urban plants and hospitals, slaughterhouses also generate wastewaters which are not usually incorporated into sewage systems. As a result, they represent a significant source of antibiotics released into the environment. The European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) collects information on how antimicrobial medicines are used in animals across the European Union (EU). According to their latest report [6], Spain is known to be one of the main consumers of veterinary drugs in the EU. As a result, many studies have monitored the occurrence of the most commonly administered pharmaceuticals in urban wastewater, groundwaters and surface water in Spain. The literature informs that concentrations of antibiotics from ng/L to µg/L [7–10]. sulfonamides [11,12], trimethoprim [13,14], β-lactams [15,16], fluoroquinolones [17,18] and macrolides [19–21] all represent a potential risk for the environment. Consequently, a representative antibiotic from each one of these groups was analyzed in this research work: sulfadiazine (veterinary use, sulfonamide), trimethoprim (human and veterinary use, trimethoprim), amoxicillin (human and veterinary use, β-lactam), enrofloxacin (veterinary use, fluoroquinolone) and azithromycin (human use, macrolide).

Among the various water treatment techniques used to eliminate these drugs, advanced oxidation processes (AOPs) are suitable for antibiotic degradation [22–24]. Other techniques, such as activated carbon or reverse osmosis, only transfer the contaminants from one phase to another without degrading them. Nevertheless, photocatalysis has been demonstrated to be effective for wastewater treatment as it is cost-effective and simultaneously oxidizes various organic contaminants into inorganic compounds, water and carbon dioxide, and pathogenic microorganisms [24]. Several semiconductors are used in photocatalysis, such as TiO₂, ZnO, and CdS. Among these, TiO₂ has been widely used because of its strong oxidizing power, availability, nontoxicity and price. The catalyst can be employed either in a colloidal or in an immobilized form. Although immobilizing the catalyst might improve the catalyst recovery, immobilized systems show lower degradation efficiencies compared to the suspended counterpart because of a reduction in the surface area [25,26]. Whenever the nanoparticles are dispersed in an aqueous medium, the depth of penetration of the radiation is limited because of absorption/scattering by the catalyst nanoparticles and the dissolved organic species. These systems also require an additional separation process to prevent Ti emission to the environment, and this stage induces further costs [27].

Pilot-scale plants represent the previous step to industrial scale plant. Literature suggests how to operate at lab-scale photocatalytic systems [28–30]. However, design and operation with a pilot-scale plant are necessary to determine how to deal with possible operational problems and establish the optimal operational parameters for real scale operation. Pilot-scale plants also allow one to determine if the real scale process would be economically feasible. Some studies about the application of TiO₂ photocatalysis in wastewater have been reported [13,14,28–31]. However, these research works do not focus on the simultaneous antibiotic removal by a TiO₂ photocatalysis pilot-scale plant applied to real wastewater.

The aim of this research work is to evaluate the presence of selected antibiotic compounds in the inlet and outlet of four WWTPs for the period of 2018–2019. Another objective is to treat selected antibiotics present in real wastewater in a photocatalytic plant by applying TiO₂ in suspension. Finally, the Ti concentration in the final effluent was controlled, to prevent Ti emission to the environment.

2. Materials and Methods

2.1. Site Description and Sample Collection

This research is focused on four WWTPs located in the POCTEFA territory (north of Spain and south of France). These WWTPs are designed to treat urban wastewater of domestic and industrial origins. Table 1 shows the main characteristics of selected WWTPs.

Samples were collected in four sampling campaigns for two years (in the spring and autumn of 2018 and 2019). The inlet and outlet of each WWTP were selected as sampling points to estimate the current removal performance of selected antibiotics in the four WWTPs, aiming to compare the different treatment lines.

Table 1. Main characteristics of each wastewater treatment plants (WWTP).

| #WWTP | Population Equivalent | Total Inlet Flow (m ³ /day) | Water Treatment Line |
|-------|-----------------------|--|--|
| 1 | 695,232 | 129,600 | Grit and grease separator/Activated Sludge/Decanter |
| 2 | 82,500 | 22,150 | Grit and grease separator/Decanter/Trickling filter (first stage) /Decanter/Trickling filter (second stage)/Decanter |
| 3 | 10,470 | 10,995 | Decanter/Trickling filter (first stage) /Decanter |
| 4 | 51,336 | 7500 | Grit and grease separator/Decanter/ Moving bed biofilm reactor/Decanter |

The sampling was carried out following the EPA method 1694 for the analysis of pharmaceuticals and personal care products in water, soil, sediment, and biosolids by liquid chromatography tandem mass spectrometry (LC/MS/MS) [32]. Amber glass bottles were used to collect 1000 mL samples which were stored under refrigeration at 4 °C. The bottles were fully filled to avoid the presence of air and properly sealed by means of a PTFE seal. According to EPA 1694, the filtration of the samples is necessary in order to remove suspension solids. Two filtration steps were carried out prior to analysis using glass fiber filters (1.6 µm, supplied by GVS) for the first filtration stage and nylon filters (0.45 µm, supplied by GVS) for the second stage, as suggested in other research works [33–35].

Mass loadings of the antibiotics were calculated in each sampling period as the product of the individual concentration of each antibiotic in the samples and the daily flow rate of each WWTP.

Removal efficiencies of the target compounds were determined as the difference between the inlet mass loading and the outlet mass loading divided by the inlet mass loading and expressed as a percentage (Equation (1)).

$$\text{Removal efficiency (\%)} : \frac{(m_{inf} - m_{eff})}{m_{inf}} \times 100 \quad (1)$$

2.2. Antibiotic Characterization

The quantification of the concentration of antibiotics was carried out via HPLC/MS/MS. Samples were centrifuged for 10 min at 13,000 rpm in Eppendorf tubes and then diluted 40-fold with 0.1% formic acid/MeOH/ACN (80%/10%/10%) before LC-MS/MS analysis. Chromatographic separations were carried out on an Ultra Performance Liquid Chromatography (UPLC) Ultimate 3000 RSLC system (Thermo Fisher Scientific). The column used was an Accucore C18 100 × 2.1 mm, 2.6 µm (Thermo Fisher Scientific). The mobile phases were A H₂O 0.4% formic acid + 5 mM ammonium formate and B MeOH/ACN 1:1 (v/v). A 20 µL sample aliquot was injected. Detection was performed on a Q Exactive Plus (Thermo Fisher Scientific) mass spectrometer operated in the targeted single ion monitoring

(SIM) positive mode with a resolution of 70,000. External calibration was used for quantification and validated by standard additions for selected samples; the samples were prepared and analyzed in triplicate. The limits of detection and quantification of each antibiotic are featured in Table 2.

Table 2. Limits of detection and quantification of selected antibiotics.

| Antibiotic | LOD (ng/L) | LOQ (ng/L) |
|--------------|------------|------------|
| Sulfadiazine | 0.8 | 2.5 |
| Trimethoprim | 0.8 | 2.5 |
| Amoxicillin | 10 | 30 |
| Enrofloxacin | 1.2 | 3.7 |
| Azithromycin | 2.0 | 6.5 |

The four antibiotics investigated in this research work were selected according to their potential risk for the environment and reported occurrence [11–21]. All of them are representative human-use and veterinary-use antibiotics belonging to the main antibiotic groups. The standards were supplied by Sigma-Aldrich. Some characteristics of the selected antibiotic compounds are included in Table 3.

Table 3. Selected antibiotics and chemical information.

| Antibiotic | Group | Chemical Abstracts Service Registry Number (CAS Nr.) | MW (g/mol) |
|--------------|-----------------|--|------------|
| Sulfadiazine | Sulfonamide | 68-35-9 | 250 |
| Trimethoprim | Trimethoprim | 738-70-5 | 290 |
| Amoxicillin | β -lactam | 26787-78-0 | 365 |
| Enrofloxacin | Fluoroquinolone | 93106-60-6 | 359 |
| Azithromycin | Macrolide | 83905-01-5 | 749 |

2.3. Total Ti Assessment in the Effluent

The effluent from the pilot-scale plant might contain some Ti which would then be emitted to the environment. The Ti concentration was quantified by an Inductively Coupled Plasma Mass Spectrometry (ICP/MS) ELAN DRC-e, PerkinElmer, Toronto, Canada. A discrete volume sample (100 μ L) was injected through a six-way valve, and the carrier was delivered directly to the nebulizer of the spectrometer. A glass concentric slurry nebulizer with a cyclonic spray chamber (Glass Expansion, Melbourne, Australia) was used. Default values were used for the rest of the instrumental parameters. The quantification of TiO₂ was based on monitoring the ICP-MS signal of the isotope ⁴⁹Ti, using ⁷⁴Ge as an internal standard. From an on-line calibration with an ionic titanium standard diluted in nitric acid (1%), intensity signals from the ICP-MS for samples were transformed into mass values by integrating the area of the transient signals obtained. All samples were injected in triplicate. The limit of detection of the method was established at 0.81 μ g/L and the limit of quantification at 2.70 μ g/L.

2.4. Photocatalytic Oxidation Experiment

The oxidation assays were carried out in the facility detailed in Figures 1 and 2. First, a 1 m³ storage tank provided with a stirrer was filled with the water sample. The solution was then pumped (8–16 L/min) to a 0.1 m³ mixer decanter where the catalyst was stored. The mixer decanter was provided with a stirrer to mix the influent with the catalyst. Subsequently, the mixture was placed in four identical slurry reactors. These reactors are made of aluminium because this material is known to have a high degree of light reflection. The reactors had a volume of 17 L and were provided with a UVA lamp (330–390 nm) of 40W. When the reactors were completely full, the UV/vis lamp in each reactor was turned on and stirring by means of compressed air took place. After treatment in the reactors, the treated water was pumped again to the decanter where a mechanical coagulation–flocculation–decantation treatment (CFD) was applied. Two steps take place in the separation process, coagulation 200 rpm

during 5 min, flocculation 40 rpm during 25 min. and 90 min of decantation, resulting in a 120 min total process. Coagulant was not added to the mechanical CFD separation process.

The effluent (clarified phase) was generated and the catalyst remained in the decanter for the next cycle.

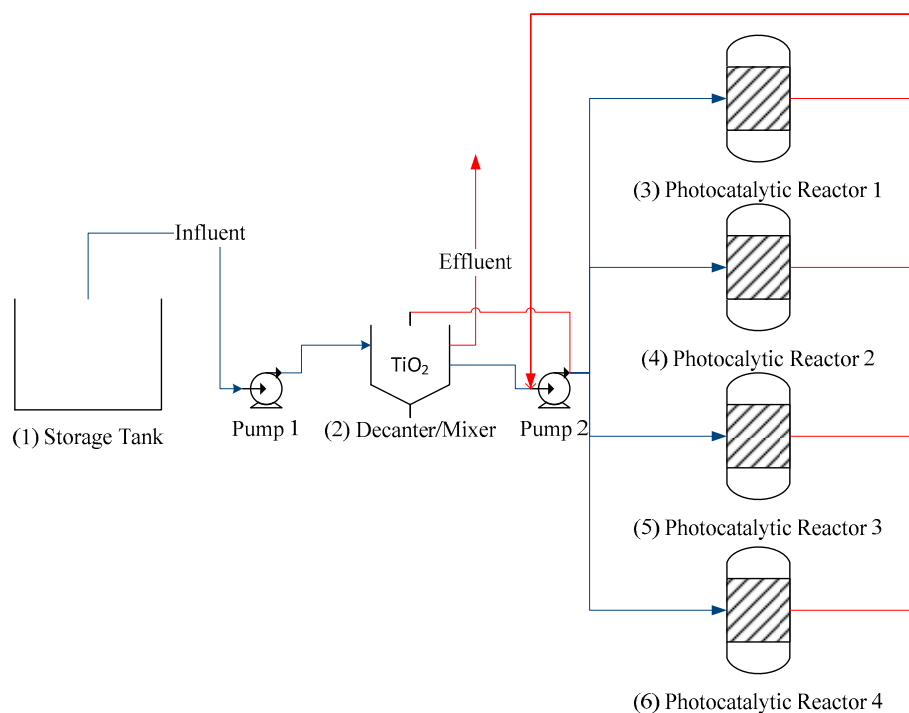


Figure 1. Pilot-scale plant process flow-diagram.



Figure 2. Pilot-scale plant process images.

The sample was prepared by the addition of individual concentrations of 1 mg/L of each antibiotic (amoxicillin, azithromycin, enrofloxacin, trimethoprim and sulfadiazine) simultaneously in the effluent from WWTP2. The physicochemical characteristics of this wastewater were pH = 7.6, DQO = 90 mg/L, Turbidity = 11 NTU. The catalyst was applied in suspension in a concentration of 1 g/L of TiO_2 FN2 (supplied by Levenger S.L.). Radiation per unity of volume was 0.3 W/L in each reactor. The temperature

ranged from 14 °C to 20 °C during the experiment. The experiments were conducted twice, and their average is represented in the results. The individual antibiotic concentration was quantified via HPLC/MS/MS during 240 min of photocatalytic treatment following the procedure described in Section 2.2.

3. Results

3.1. Occurrence of Target Antibiotics in Urban Wastewaters

Figure 3 shows the mass loading of the selected antibiotics at the four WWTPs during 2018 and 2019. It should be noted that atypical points are not represented. However, they are all available in Tables S1 and S2 of the Supplementary Material. The tables show that the highest mass loading corresponds to azithromycin. This human-use antibiotic presented an average load of around 925 mg/day per 1000 inhabitants and reached a maximum load of 11,332 mg/day per 1000 inhabitants in WWTP4. The azithromycin concentration increased over the four sampling campaigns in all the WWTPs. This fact might be attributed to a major increase in the use of azithromycin [36]. Enrofloxacin (fluoroquinolone group) also followed the same trend: higher loads were detected over the campaigns analyzed in this study. This veterinary-use antibiotic showed median mass loadings of around 200 mg/day per 1000 inhabitants and a maximum of 4329 mg/day per 1000 inhabitants. The enrofloxacin mass load was higher in the spring of both years, while it was rarely found in autumn, and always in lower loads, reflecting a seasonal use of this fluoroquinolone. The seasonal appearance of enrofloxacin has also been recently reported in another research work [37].

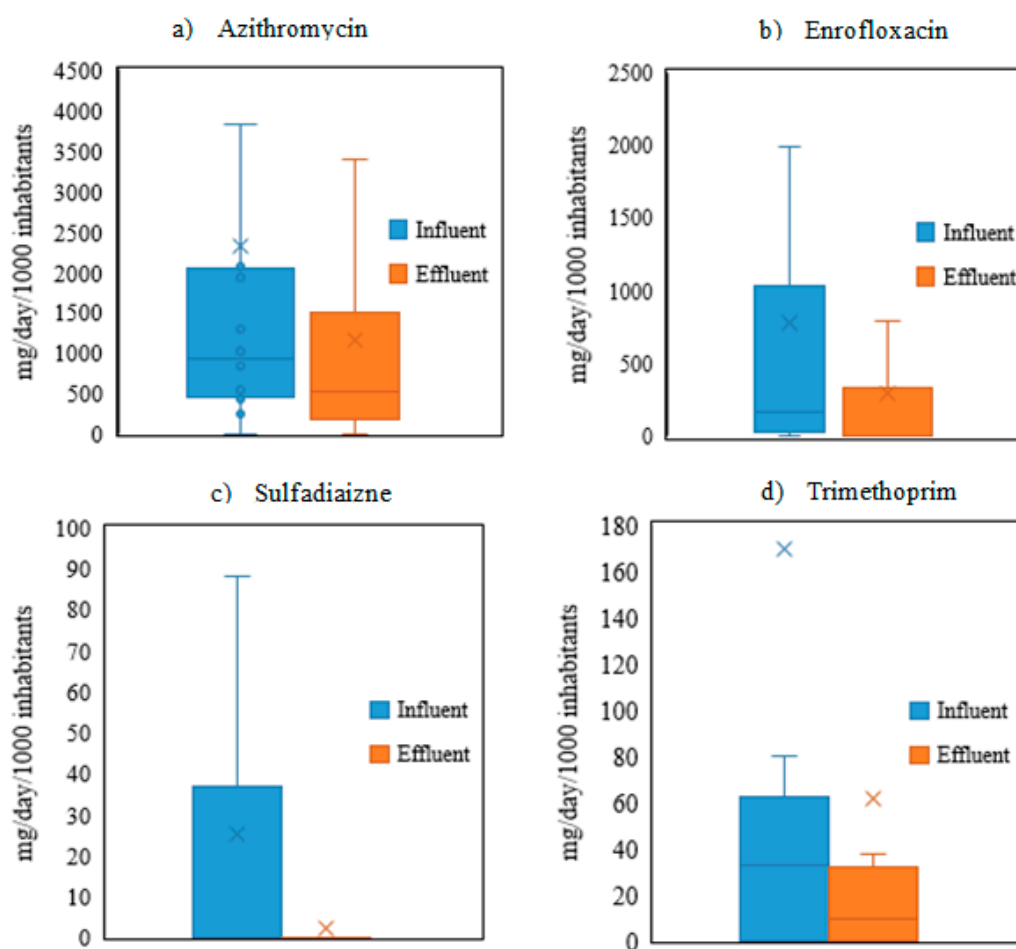


Figure 3. Boxplots of each antibiotic indicating total mass loading values: (a) azithromycin, (b) enrofloxacin, (c) sulfadiazine (d) trimethoprim.

By contrast, the average mass loadings of the other two antibiotics (trimethoprim and sulfadiazine) ranged from 35 mg/day per 1000 inhabitants for trimethoprim, to undetectable levels for sulfadiazine. Comparing the median mass loadings of trimethoprim and sulfadiazine with enrofloxacin and azithromycin reveals a difference greater than one order of magnitude.

Amoxicillin was not found in any sample (influent and effluent of the WWTPs). This fact could be attributed to the low stability of amoxicillin and the subsequent generation of degradation products such as amoxicillin penicilloic acid or amoxicillin-diketopiperazine-2', 5', as is suggested in the literature [38–40]. These degradation products were found in subsequent campaigns carried out in the same sampling points.

Finally, Tables S1 and S2 show that the reported load of each antibiotic varies significantly, by more than one order of magnitude, between the years and seasons. This trend could be related to the differences in rainfall patterns between the two years: high flows in particular were reported in spring 2018 and in autumn 2019 in both the Ebro River basin and the Cantabrico Occidental River basin. However, the total mass loading of the four antibiotics was relatively higher in the spring: $\sum_{\text{SPRING}} = 69.4$ g/day per 1000 inhabitants versus $\sum_{\text{AUTUMN}} = 13.6$ g/day per 1000 inhabitants.

3.2. Removal Efficiency of Selected WWTPs

The results of the removal efficiency of the antibiotics in each WWTP is shown in Figure 2. These results are also fully detailed in Table S3. Figure 4 shows that the removal efficiencies range from 2%–100%, demonstrating the fact that WWTPs can partially or almost totally remove the target antibiotics.

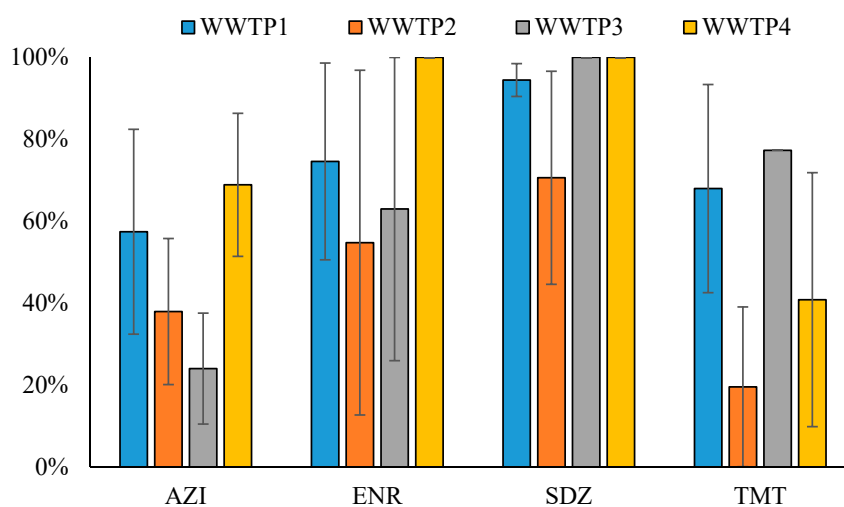


Figure 4. Wastewater treatment plants' removal efficiency for each antibiotic in 2018–2019 (AZI = azithromycin, ENR = enrofloxacin, SDZ = sulfadiazine, TMT = trimethoprim).

The results also suggest a significant variation for each antibiotic in each WWTP, indicating that the removal efficiency strongly depends not only on the specific matrix but also on the season and associated flow. This variation might also be attributed to the different physicochemical characteristics of the antibiotics, such as the degradation rates in the water, organic carbon–water partition coefficients or acid dissociation constants and water solubilities. More precisely, the results suggest that sulfadiazine is the antibiotic with the highest removal efficiency in every case. This trend may be due to the low mass loadings of this antibiotic, which make its removal easier [41]. Enrofloxacin also presents a high removal efficiency after the wastewater treatment, reaching 100% in several samples. By contrast, azithromycin and trimethoprim showed lower removal efficiencies in all the WWTPs. It should be noted that azithromycin had the highest mass loadings, more than one order of magnitude greater than the other antibiotics. It might be also attributed to the fact that azithromycin and trimethoprim

have similar carbon–water partition coefficients [42]. Some studies suggest that biological processes, which are present in the four WWTPs, can remove fluoroquinolones effectively, while trimethoprim is more difficult to remove by means of biological treatments [43].

Comparing the different treatments of each WWTP, it can be observed that the facilities provided with a trickling filter (WWTP2 and WWTP3) showed higher removal efficiencies of the target antibiotics. This is consistent with some studies which demonstrate that trickling filters can remove antibiotics and other pharmaceuticals as well as personal care products [44–46]. WWTP4 showed the highest average antibiotic removal in most samples. However, it is important to note that this WWTP is in the Cantabrico River basin where there is higher rainfall than in the Ebro River Basin. As a result, the selected antibiotics had lower mass loadings in WWTP4.

3.3. Photocatalytic Oxidation of Antibiotics

Figure 5 shows the performance of the photocatalytic assays for the simultaneous oxidation of amoxicillin, enrofloxacin, sulfadiazine, trimethoprim and azithromycin during 4 h of treatment. The results show that in only 30 min of treatment, azithromycin and amoxicillin reached a degradation rate of 85% and 75%, respectively. Moreover, after 120 min of treatment, both antibiotics were completely removed from the wastewater. Amoxicillin was previously reported to be easily removed from waters by TiO_2 photocatalysis applied to the isolated compound at lab scale [47]. However, sulfadiazine shows a slower degradation rate, achieving degradation yields of 25% and 100% in 30 min and 240 min, respectively. In contrast, enrofloxacin and trimethoprim were not completely removed from the wastewater after the treatment. The degradation rate of enrofloxacin was relatively high at the beginning of the process (degradation yield of 50% in 30 min of treatment), but complete degradation was not achieved by the end of the treatment. A similar trend has been reported in other research work at lab scale [31]. Finally, trimethoprim showed the slowest initial degradation rate during the first 120 min, as reported in other studies at lab scale [45], while its degradation yield at the end of the treatment was close to 70%.

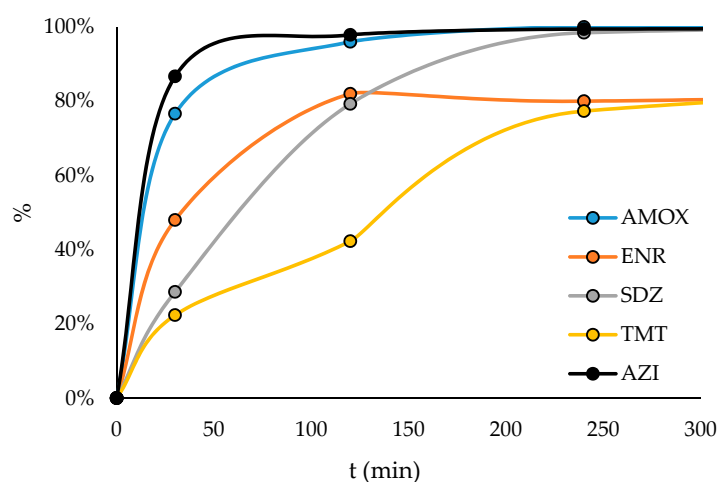


Figure 5. TiO_2 photocatalysis degradation yields of each antibiotic in WWTP4 during 240 min of treatment (individual antibiotic initial concentration = 1 mg/L, TiO_2 initial concentration = 1 g/L, radiation per unity of volume = 0.3 W/L). AMX = amoxicillin, AZI = azithromycin, ENR = enrofloxacin, SDZ = sulfadiazine, TMT = trimethoprim.

3.4. Ti Assessment in the Effluent of the Photocatalytic Treatment Plant

In order to determine whether Ti is released into the environment, a quantification of the Ti concentration in the effluent was carried out applying a coagulation–flocculation–decantation treatment. TiO_2 has been demonstrated to be effective after several cycles of photocatalytic treatment for the degradation of pharmaceuticals [48], so it is important to recover it effectively. Fortunately, the results of

the Ti assessment suggest that no more than 0.1% of the initial Ti concentration remained in the effluent. This reflects the fact that recovery by means of mechanical coagulation–flocculation–decantation treatment is quite efficient for recovering TiO₂ when used in suspension [48].

4. Conclusions

This research work evaluates the behavior of four antibiotic compounds in four different WWTPs located in the north of Spain. The mass loadings of amoxicillin, enrofloxacin, sulfadiazine, trimethoprim and azithromycin were analyzed in the influent and effluent of the WWTPs. The performance of a TiO₂ photocatalytic treatment plant applied to the simultaneous removal of the antibiotics from real urban treated water was evaluated. This showed that the technology can be used to totally remove some of the selected antibiotics at slightly higher concentrations than those commonly found in wastewaters. The conclusions can be summarized as follows:

1. The mass loadings of the antibiotics ranged from 11,332 mg/day·1000 inhabitants to undetectable levels. Azithromycin had the highest mass loadings, followed by enrofloxacin, trimethoprim, sulfadiazine and amoxicillin.
2. The use of enrofloxacin and azithromycin increased in the locations of the WWTPs during the period of this study.
3. Sulfadiazine, amoxicillin and azithromycin were totally removed from wastewaters in the TiO₂ photocatalytic pilot-scale plant, while 80% removal of trimethoprim and enrofloxacin was achieved by the treatment. Moreover, the facility was able to recover the catalyst after the treatment, minimizing the Ti released into the environment and allowing catalyst reuse.
4. Although WWTPs are not designed to remove antibiotics, they do reduce them. This research shows that biological treatments have a significant influence on antibiotic removal. In particular, the presence of a trickling filter in the water treatment line of the WWTPs has been demonstrated to lead to a higher degree of antibiotic removal. However, the efficiency of the antibiotic removal depends on the physicochemical properties of the antibiotics and on the characteristics of the wastewater.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2073-4441/12/5/1453/s1>, Table S1: Spring and autumn 2018 Load selected antibiotics in mg/day/1000 inhabitants. Table S2: Spring and autumn 2019 Load selected antibiotics in mg/day/1000 inhabitants. Table S3: Removal efficiencies of the different WWTPs for selected antibiotics.

Author Contributions: Planning photocatalysis experimentation, R.M. and S.M.; general experimental work coordinator, M.P.O.; LC/MS/MS analysis, S.G. (technician) and J.S. (responsible); design and construction of pilot-scale plant, J.G. and S.M.; responsible of nanoparticles detection in the effluent, J.R.C.; experimental photocatalysis technician and principal author, S.M. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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