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Antibiotics in the Environment: Occurrence in Italian STPs, Fate, and Preliminary Assessment on Algal Toxicity of Amoxicillin

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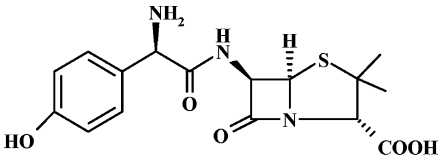
Amoxicillin is a widely used penicillin-like antibiotic, and due to its presence in several effluents of ItalianSTPs, its environmental fate along with its toxicity toward simple organisms have been investigated in model conditions. The present study shows that under abiotic conditions both hydrolysis and direct photolysis could be responsible for the transformation and removal of amoxicillin in aquatic environment, especially in slightly basic media. Quantum yields for the solar direct photolysis have been calculated along with kinetic constants and half-life times. Indirect photolysis experiments in the presence of natural photosensitizers such as nitrate ions and humic acids indicate that nitrate ions have no influence on the photodegradation rate of amoxicillin, while humic acids are able to enhance it. Standard batch experiments have been also performed under biotic conditions. The results indicated that also biodegradation on activated sludge is an effective pathway through which amoxicillin can be removed from the aquatic environment. Rate constants for biodegradation and adsorption have been calculated by applying simple pseudo-first-order kinetic models. Algal bioassays indicate that, in the range of concentrations from 50 ng/L to 50 mg/L, amoxicillin is not toxic toward eucariotic organisms such as the Chlorophyceae *Pseudokirkneriella subcapitata* and *Closterium ehrenbergii* and the Bacillariophyceae *Cyclotella meneghiniana*, but it shows a marked toxicity toward the Cyanophyta *Synechococcus leopolensis*.

Introduction

Amoxicillin (**1**) is a broad spectrum aminopenicillin antibiotic, widely used in human and veterinary medicine. The interest



(1)

to this molecule arises from the emerging problem of the presence of pharmaceuticals in different environmental compartments and the potential toxicity of this kind of active substances on living organisms (*1*−*6*). Like other biologically active substances, pharmaceuticals can enter wastewater as parent compounds or metabolites mainly via human excretion. Additional sources of drug pollution are represented by improper disposal of expired medications and agricultural and aquacultural activities, the latter being particularly relevant in the case of amoxicillin (*7*). Several studies (*8*, 9) have demonstrated that removal of pharmaceuticals in sewage treatment plants (STPs) is often incomplete, so they are discharged into surface waters and from there enter different aquatic compartments (e.g., groundwaters, drinking waters). Concerning antibiotics one important aspect related with their presence in the environment is the possibility of inducing resistance in bacterial strains (*2*, *3*, *10*, *11*), namely, a genetic selection of resistant bacteria, an irreversible effect favored by low concentrations of the antibiotic as in the case of environmental pharmaceuticals concentrations (*12*). There is evidence that this resistance can pass to humans, in part via environmental exposure (*13*) even if this subject is still under debate (*14*). Furthermore, the (bio)accumulation of small quantities of antibiotics may be cause of serious problems for humans and animals on a long time span. Once released into the environment, a pharmaceutical is characterized by a certain persistence in nature, depending on the structure of the compound and on the properties of the particular environment. The knowledge of the behavior of the molecule investigated under natural conditions, along with its toxicity toward living organisms, is fundamental to predict the environmental risk. Efforts in this direction have been made in the past few years by many research groups that, following the experimental rules stated by the Environmental Protection Agency (EPA) and the Organisation for Economic Co-operation and Development (OECD), have carried out model studies to predict the persistence in the environment of various chemicals (*5*, *15*−*18*). Fortunately, biotic and abiotic processes responsible for transformation and removal of organic compounds normally occur in the environment. The former involve microorganisms mediated degradation of the organic compound (*19*), while the two major abiotic processes are photolysis and hydrolysis. Photolytic degradation processes in the environment are caused by absorption of solar light by chemical species (direct photolysis; *20*), or are mediated by natural photosensitizers such as nitrate and humic acids (indirect photolysis; *21*). Pharmaceuticals, because of the presence in their structure of various functional groups, are expected to be susceptible to undergo many indirect photodegradation pathways such as reactions with oxygen reactive species (singlet oxygen, peroxy and hydroxyl radicals) and also with photoexcited organic matter. Under solar irradiation nitrate ions and humic acids, always present in natural waters, can give rise to oxygen reactive species or to photoexcited species (*22*, *23*) that can in turn react with the organic compound, or transfer it energy, so enhancing its photodegradation rate. Humic acids can

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also show in some case an opposite effect on the photodegradation rate as they can absorb solar light acting as inner filters toward the molecule under investigation (*24*). In this case, the photodegradation rate is lower in the presence of humic substances. Another aspect that has to be considered concerning the photodegradation rate of a chemical is the dependence of solar irradiance on both latitude and season. This implies a strong variation of the half-life time in water among different places in the world and ultimately of the persistence of the compound. Concerning hydrolysis, many pharmaceuticals are designed for oral intake and for this reason are not susceptible of this reaction. Amoxicillin belongs to a group of drugs excreted unmetabolized and has been chosen as subject of this study because of its heavy predicted environmental load, as it is one of the most saled and used antibiotics in Italy (*6*), Germany (*3*), U.K (*25*), and The Netherlands (*7*) both for human and veterinary purposes. On the other hand, the real difficulty to detect amoxicillin in natural waters is due to its instability toward extractive workup from environmental samples (*3*) or to the too low sensitivity of the analytical methods (*6*). The present study shows the results of a monitoring campaign on amoxicillin in several Italian STP effluents and on the persistence of amoxicillin in aquatic environment under both abiotic and biotic conditions, through the determination of kinetic parameters such as experimentally determined quantum yield of direct photolysis, biodegradation, and adsorption rate constants. Preliminary results of toxicity on simple organisms such algae are also reported, with the aim of assessing the environmental risk of this antibiotic.

Experimental Section

Monitoring Campaign: Analytical Methods and Instrumentation. Amoxicillin was detected in the frame of an analytical campaign aimed to measure pharmaceuticals in the effluents of eight STPs distributed over Italy. Composite water samples (2.5 L each, pooling 5 aliquots collected from the same site with 1-h intervals) were collected from effluents of the STPs in Cagliari (Cagliari Is Arena), Cosenza (Settimo di Montalto Uffugo), Palermo (Acqua dei Corsari), Roma (Latina), Napoli (Cuma), Torino (ACDA Cuneo), Varese Olona (Pravaccio), and Varese Lago (Gavirate). Amoxicillin was extracted from water with a 3-mL Bakerbond C_{18} cartridge (500 mg, Baker, Phillipsburg NJ). Samples were analyzed with a triple quadrupole HPLC-MS−MS system consisting of two Perkin-Elmer series 200 pumps, a Perkin-Elmer series 200 autosampler, and an API 3000 mass spectrometer (Applied Biosystem-Sciex, Toronto, Canada). The mass spectrometer was equipped with a standard turbo ion spray ionization source. HPLC separation was done with a LUNA C8 column 50 mm × 2 mm i.d., 3 μm particle size (Phenomenex, Torrance, CA). Amoxicillin was detected in positive ionization mode using salbutamol-d_6 as internal standard. Data acquisition was done in the multiple reaction monitoring mode to achieve maximum sensitivity. Details of the methods are provided elsewere (*6*).

Abiotic and Biotic Processes: Chemicals and Instrumentation. Amoxicillin (**1**) was purchased from ICN Biochemicals Inc.; *p*-nitroacetophenone (PNAP) was supplied by Aldrich while pyridine was purchased from Riedel-de-Haen. Humate sodium salt (from Aldrich) was used as substitute for aquatic humic acids (*21*). Other reagents used were of p.a. grade or better. Acetonitrile (Aldrich), methanol (Lancaster), and water (Aldrich) were of HPLC grade. HPLC analyses were performed on a 1100 series Hewlett-Packard instrument, equipped with an UV−Vis diode array detector, using a reversed-phase column (RP-C_6 Phenomenex, 250 mm × 3.2 mm i.d.). The UV spectra were recorded on a UV−Vis spectrophotometer (HP 8452 A) using quartz cells (path length = 1 cm). The average molar absorbivities ($M^{-1}\cdot cm^{-1}$) were calculated for amoxicillin from the UV absorbances of three different standard solutions (0.025, 0.05, or 0.1 mM). An Orion 960 pH-meter with a glass pH electrode was used for pH measurements, and the pH was adjusted by adding diluted $HClO_4$ or NaOH. See Supporting Information for analytical details on HPLC analyses and details about biodegradation experiments.

Abiotic Processes. (a) Hydrolysis Experiments. Two solutions of amoxicillin in bidistilled water (0.05 mM) were prepared, taken to pH 5.5 and pH 7.5, and kept in test glass tubes (e.d. = 1.3 cm; i.d. = 1.1 cm; V = 10 mL) covered by aluminum foil. The temperature was maintained constant at 25 °C by means of a water bath. Aliquots of each sample were withdrawn at appropriated intervals and analyzed by HPLC in order to monitor the substrate decay.

(b) Direct Photolysis Experiments. Two solutions of amoxicillin in bidistilled water (0.05 mM) were prepared and taken to pH 5.5 and pH 7.5, respectively, before being exposed to natural sunlight at Naples (37° N, 14° E), Italy. The same irradiation experiment was started on a solution 0.02 mM in bidistilled water of the standard actinometer PNAP containing 120 mM pyridine (*27*). The concentration of pyridine was chosen in order to obtain a PNAP decay similar to that of amoxicillin in the same irradiation period (*26*). All samples were contained in 0.5-L glass reactors, and the temperature was kept constant at 25 °C by means of a water bath during the irradiation period. Aliquots of all samples were withdrawn at appropriate intervals and separately analyzed by HPLC to monitor the decay of amoxicillin and PNAP.

(c) Indirect Photolysis. Two solutions of amoxicillin in bidistilled water (0.05 mM) containing 15 mg/L of nitrate ions and 5 mg/L of humic acids, respectively, were prepared (*26*). Each solution was divided into two aliquots, and each aliquot containing the appropriate photosensitizer was taken to pH 5.5 and pH 7.5 before being exposed to natural sunlight at Naples (37° N, 14° E), Italy. Blank solutions of amoxicillin in bidistilled water (0.05 mM) were kept at pH 5.5 and pH 7.5, respectively, and exposed to natural solar light during the same period. All samples were contained in 0.5 dm^3 glass reactors, and the temperature was maintained constant at 25 °C by means of a water bath during the irradiation period. Aliquots of each sample were withdrawn at appropriate intervals and analyzed by HPLC.

Biotic Processes. The experiments described in this section were performed by using activated sludge collected from the municipal wastewater treatment plant of Cuma (Naples), which receives predominantly domestic sewage. In all cases before the experiments, the sludge was preconditioned by aeration under stirring for 1 night. All batch experiments were performed in triplicate. The experiments were carried out following the OECD protocol (*28*).

Biodegradation Batch Experiments. A 125-mL suspension of activated sludge (mixed liquor suspended solids: 6.0 g/L) was diluted in 125 mL of bidistilled water containing the appropriate quantities (*28*) of mineral nutrients and spiked with 4.6 mg of amoxicillin to obtain a nominal concentration of 0.05 mM (18 μg/L). The slurry was aerated and stirred over the whole investigation period in order to ensure aerobic conditions and homogeneity, and the temperature was maintained constant at 25 °C by means of a water bath. All samples were contained in 250-mL glass flasks. Aliquots of the homogeneous suspension were withdrawn at different reaction times and filtered on cellulose disks before the HPLC analysis to monitor the substrate decay. To get the real kinetic of the biodegradation process, it was necessary to allow the microorganisms to acclimate to amoxicillin. This goal was achieved by means of the following procedure. A solution containing the mineral medium, and amoxicillin at a concentration of 0.05 mM was added with a fixed amount of sludge, aerated, and stored at constant temperature (25

°C). The system evolution was followed until the almost complete disappearance of amoxicillin was observed. After the aeration and stirring were stopped, the sludge was allowed to settle. An appropriate volume of the supernatant liquid was drawn off, and the flask was refilled to the original volume with aqueous solution containing the mineral medium and amoxicillin at the right concentrations to restore the initial conditions.

The system was newsly aerated and stirred. This procedure was repeated until the recorded amoxicillin concentrations against time did not show any significant difference with respect to the substrate decay found before the last reloading of the pharmaceutical to the flask.

Adsorption Experiments. A 50-mL suspension of activated sludge (mixed liquor suspended solids: 6.0 g/L) was diluted in 50 mL of bidistilled water containing the appropriate quantities of mineral nutrients and then treated with $HgCl_2$ (100 mg/L) (*28*). The resulting suspension was agitated and aerated for 2 h before adding amoxicillin (nominal concentration 0.05 mM) and starting the experiment. The slurry was aerated and stirred over the whole investigation period in order to ensure aerobic conditions and homogeneity, and the system was maintained at a constant temperature of 25 °C by a water bath. The samples were contained in 100-mL glass flasks. Aliquots of the homogeneous suspension were withdrawn at appropriate intervals and filtered on cellulose prior being analyzed by HPLC to monitor the substrate behavior.

Toxicity Experiments. The algae tested against amoxicillin were the Chlorophyceae *Psudokirkneriella subcapitata* (strain UTEX 1648), *Closterium ehrenbergii* (strain NIES 228), the diatom *Cyclotella meneghiniana* (strain SAG 1020-1a), and the Cyanophyte *Synechococcus leopolensis* (strain UTEX 625). Inocula corresponding to 10 000 or 100 000 cells/mL from cultures in mid-exponential phase were grown in 100-mL Erlenmeyer flasks, under a continuous light, with a total irradiance of 100 μEinstein·m^{-2}·s^{-1} provided by daylight fluorescent Philips lamps (TLD 30 w/55). *Closterium ehrenbergii* was cultured in 12 well-plates (*29*) at the same light intensity. All the Chlorophytes were cultured at 23 ± 1 °C, whereas *S. leopolensis* and *C. meneghiniana* were grown at 28 ± 1 °C. The algal growth was followed after 96 h from the addition of amoxicillin. Amoxicillin was added to give final concentrations ranging from 50 ng/L to 50 mg/L. The experiments were carried out in quadriplicate, and the results were evaluated on the basis of three tests. The growth of algae was followed as absorbance increase at 550 nm, with a colorimeter Baush & Lomb Spectronic 20. The growth of *C. ehrenbergii* was followed by counting the cells in each well (*30*) with an inverted microscope Leitz Diavert. In each 96-h bioassay, the relative EC_{50}, used as acute measurement end point, was determined by regression using log−logistic models, as previously reported (*31*). Chronic NOEC (no observed effect concentration) and LOEC (lowest observed effect concentration) were determined by hypothesis tests. Dunnett's tests after verifying the Shapiro−Wilk's test for normality and the Hartley test for homogeneity of variance were used. Calculations were performed using TOXSTAT 3.0 software (*32*).

Results and Discussion

Monitoring Campaign Results. The mean recovery of amoxicillin from water (10 ng/L in 100 mL) was 50%. The correlation coefficient (calculated in the range between 0.1 and 0.01 ng/μL) was 0.98. The limits of quantification (LOQ) was 1.8 ng/L. Further details of the results of the method are provided elsewere (*6*). Concentrations of amoxicillin in effluents of the STPs analyzed ranged from undetectable (<1.80 ng/L) to about 120 ng/L: Cagliari, 7.40 ng/L; Cosenza, <1.80 ng/L; Palermo, 120.35 ng/L; Roma, 15.20 ng/L; Napoli, 1.80 ng/L; Torino, 4.74 ng/L; Varese (Olona), 4.68 ng/L; Varese (Lago), 4.68 ng/L.

TABLE 1. Comparison of Molar Absorption Coefficients of Amoxicillin for $\lambda > 290$ nm

λ (nm)	ϵ (M^{-1} cm^{-1}) [a]	ϵ (M^{-1} cm^{-1}) [b]	λ (nm)	ϵ (M^{-1} cm^{-1}) [a]	ϵ (M^{-1} cm^{-1}) [b]
297.5	57.4	218	312.5	43.8	154
300	53.8	204	315	43.2	147
302.5	51.3	186	317.5	42.6	102.4
305	48.8	179	320	42.2	140
307.5	47.0	168.9	323	41.6	138
310	45.2	159	330	41.4	132

[a] pH 5.5. [b] pH 7.5.

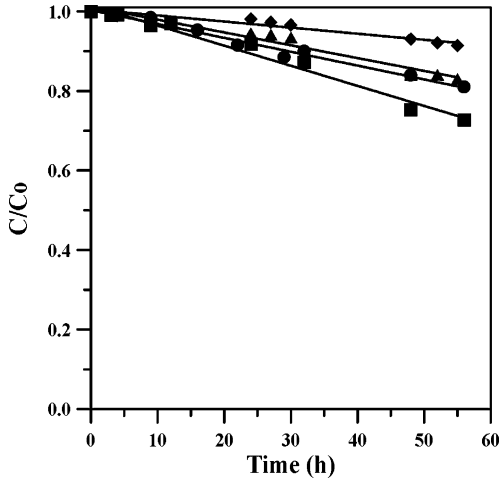

FIGURE 1. Hydrolysis (◆, pH 5.5; ▲, pH 7.5) and direct photolysis (●, pH 5.5; ■, pH 7.5) of amoxicillin in bidistilled water. $C_o = 0.05$ mM. $T = 25$ °C.

Abiotic Processes. (a) Hydrolysis and Direct Photolysis. On the basis of the chemical structure of amoxicillin (**1**) and of its UV absorption data for $\lambda > 290$ nm at investigated pH values (Table 1), the tendency of amoxicillin to undergo both hydrolysis and direct photolysis can be predicted.

The hydrolysis reaction would proceed through the attack of the nucleofile H_2O to the β-lactam ring followed by ring opening, whereas the UV absorption data of amoxicillin at pH 5.5 and pH 7.5 show that the molecule absorbs solar radiation for $\lambda > 290$ nm and thus could be susceptible of photoinduced transformations. The results of hydrolysis and direct photolysis experiments at the investigated pH values are shown in the Figure 1. Both processes are favored in slightly basic aqueous medium. In the case of photolysis, this is probably due to the fact that at pH 7.5 the molar absorption coefficients of the molecule are slightly higher than at pH 5.5 (see Table 1).

The experimental data obtained in dark experiments were used to calculate the hydrolysis rate constants of amoxicillin at pH 5.5 and pH 7.5, by using a pseudo-first-order kinetic model. The values of $k_{hydr} = 1.46 \times 10^{-3}$ (h^{-1}) at pH 5.5 and $k_{hydr} = 3.17 \times 10^{-3}$ (h^{-1}) at pH 7.5 have been obtained, respectively. The rate constants for the direct photolysis process have been calculated by a pseudo-first-order kinetic model, which takes into account the hydrolysis contribution to the loss of substrate during the irradiation period:

$$\frac{d[A]}{dt} = -k_{hydr}[A] - k_{phot}[A]$$

The values so calculated are $k_{phot} = 2.09 \times 10^{-3}$ (h^{-1}) at pH 5.5 and $k_{phot} = 3.09 \times 10^{-3}$ (h^{-1}) at pH 7.5.

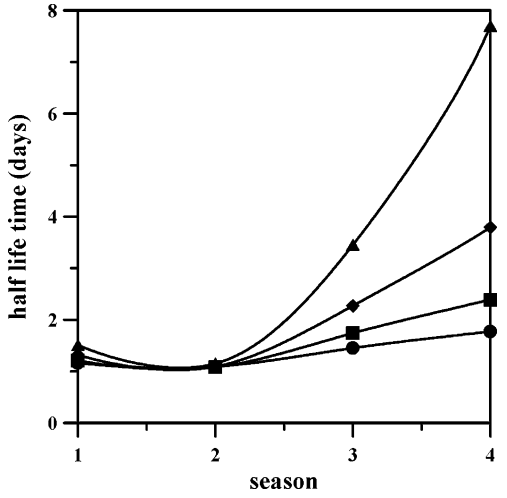

FIGURE 2. Half-life times for direct photolysis of amoxicillin in bidistilled water at pH 5.5, at varying season and latitude: (1) spring, (2) summer, (3) fall, and (4) winter (●, 20° N; ■, 30° N; ◆, 40° N; ▲, 50° N).

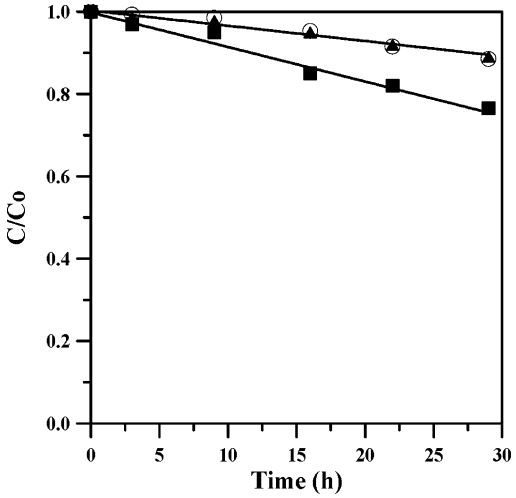

FIGURE 3. Photodegradation at pH 5.5 of amoxicillin under sunlight in bidistilled water (○), with 15 mg/L of NO_3^- (▲), and with 5 mg/L of humic acids (■). $C_0 = 0.05$ mM. $T = 25$ °C.

(b) Determination of Quantum Yields and Half-life Times for Direct Photolysis. A measure of the tendency of amoxicillin to undergo direct photolysis is given by the quantum yields calculated from the data obtained during the irradiation experiments of both amoxicillin and PNAP, using the following equation:

$$\Phi_{amox} = \Phi_{act}\frac{k_{phot}}{k_{act}}\frac{\sum_\lambda(\epsilon_\lambda L_\lambda)_{act}}{\sum_\lambda(\epsilon_\lambda L_\lambda)_{amox}}$$

where k_{phot} is the calculated direct photolysis rate constant for amoxicillin reported in the previous section and k_{act} (4.65×10^{-3} h^{-1}) is the rate constant for the direct photolysis of the standard solution of the actinometer, calculated from the PNAP solar experiment data; Φ_{act} is the actinometer quantum yield of direct photolysis, evaluated by the equation $\Phi_{act} = 0.0169[py]$, where [py] represents the concentration of pyridine added to the solution (*26*); ϵ_λ ($M^{-1}\cdot cm^{-1}$) is the molar absorption coefficient at the wavelength λ; and L_λ (Einstein$\cdot cm^{-2}\cdot d^{-1}$) is the average daily value for irradiance at the wavelength λ. In Table 1 the measured average ϵ_λ values for amoxicillin at pH 5.5 and pH 7.5 are shown. The values of L_λ and of $\Sigma_\lambda(\epsilon_\lambda L_\lambda)_{act}$ for different seasons and decadic latitudes are reported in the literature (*20*, *26*).

It is noteworthy to observe that—being amoxicillin capable of giving rise to the formation of different species according to the pH of the solution and p*K* values of amocillin itself—Φ_{amox}, which is calculated by using the above-reported equation, is just a conditional quantum yield of the mixture. That is it reflects the capability to undergo photolytic reaction of different forms in which amoxicillin is present in the solution whose occurrence is regulated by pH.

The values of quantum yields for the direct photolysis of amoxicillin (Φ_{amox}) calculated under solar irradiation are 5.97×10^{-3} at pH 7.5 and 4.47×10^{-3} at pH 5.5. Once the quantum yields were calculated at both investigated pH values, half-life times ($t_{1/2}$) of amoxicillin in different conditions of season and latitude were determined using the following equation:

$$t_{1/2} = \frac{\ln 2}{\Phi_{amox}\sum_\lambda(\epsilon_\lambda L_\lambda)_{amox}}$$

Predicted values for spring at Naples, 37° N latitude, are 1.13 d at pH 7.5 and 1.69 d at pH 5.5. In Figure 2, the half-life times for the direct photolysis of amoxicillin in bidistilled water at pH 5.5 are plotted for different seasons, at varying the latitude. It can be observed that amoxicillin is characterized by a maximum half-life time of about 9 d in winter at 50° latitude, while the minimum is of about 2 d in summer at 20° latitude. (The results obtained at pH 7.5 are reported in the Supporting Information.)

(c) Indirect Photolysis. The influence of nitrate ions and humic acids on the photodegradation rate of amoxicillin at pH 5.5 can be derived from the diagrams in Figure 3. Similar results have been obtained at pH 7.5 (data not shown). The effect of nitrate ions is negligible at both investigated pH values. This could be explained by the fact that nitrate ion absorbs sunlight as amoxicillin in the middle-UV region (*33*), and this overlapping could result in a reduction of its rate of hydroxyl radicals production. On the other hand when humic acids are presents, the photodegradation rate of amoxicillin is enhanced both at pH 5.5 and pH 7.5, in agreement with previously reported results (*5*, *16*).

Biotic Processes. (a) Biodegradation and Adsorption on Activated Sludge. The environmental fate of amoxicillin has been investigated by performing batch experiments of biodegradation and adsorption on sludge with the aim of evaluating the rate constants for both processes. Indeed the removal of chemicals from the environment by microorganism mediated degradation or by adsorption of the compound on activated sludge is an important pathway in natural environment. Furthermore, the values of the rate constants for biodegradation and adsorption on sludges and sediments of a chemical influence the predicted environmental concentration (PEC) of a substance and its environmental risk assessment. Figure 4 shows the biodegradation profile (black symbols) obtained during a typical experiment. Interestingly no "lag phase" was observed, also in the presence of non-pre-acclimatated sludge. This is probably due to the structural similarity between amoxicillin and other natural substances (e.g., penicillins) that renders the molecule "familiar" to microorganisms. Adsorption experiments were carried out by using $HgCl_2$ as sterilizing agent in order to inactivate the microorganisms and thus inhibiting the biodegradation process. The experimental profile of adsorption is shown in the Figure 4 (open circles). From obtained results it is evident that both biodegradation and adsorption processes can play an important role in the transformation and removal of amoxicillin from the aquatic environment, the former being the faster one.

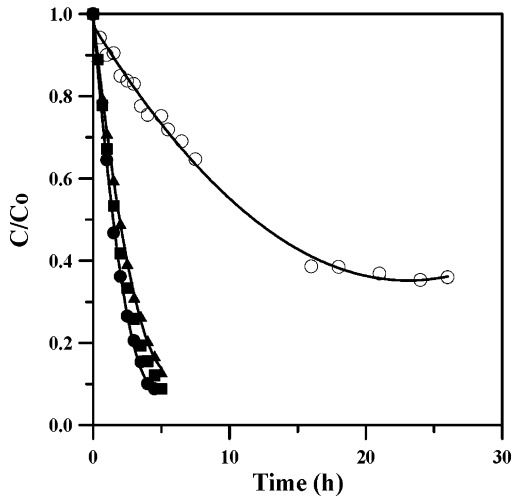

FIGURE 4. Adsorption and biodegradation of amoxicillin on activated sludge (3 g/L). C_0 = 0.05 mM. T = 25 °C. Adsorption (○), biodegradation (●, III reload; ■, IV reload; ▲, V reload).

Kinetic Model. The following kinetic model was proposed for adsorption and biodegradation processes, respectively:

$$A + s^* \underset{k_1}{\overset{k_{-1}}{\rightleftharpoons}} A_{ads} \qquad (a)$$

$$A \xrightarrow{k_{bio}} \text{products} \qquad (b)$$

When biodegradation is prevented by $HgCl_2$ addition, if a significant physical adsorption of amoxicillin occurs on s* sites of the sludges, the following mass balance can be written for the substrate A:

$$\frac{d[A]}{dt} = -k_1[A][s^*]Q + k_{-1}[A_{ads}]Q \qquad (1)$$

where Q is the load of suspended sludges (in the present case 3.0 g·L^{-1}) and $[A_{ads}]$ represents the concentration of adsorbed substrate A. The concentrations of s* sites and adsorbed species are expressed as moles/mass of sludge. Since $[A_{ads}]\cdot Q = [A]_0 - [A]$, where $[A]_0$ is the initial concentration of substrate A, and considering the concentration [s*] constant, the eq 1 can be written as:

$$\frac{d[A]}{dt} = -k'_1[A] + k_{-1}([A]_0 - [A]) \qquad (2)$$

where $k'_1 = k_1[s^*]Q$.

When equilibrium (a) is achieved ($d[A]/dt = 0$), eq 2 can be transformed as follows:

$$\frac{d[A]}{dt} = -k_{-1}R[A] + k_{-1}([A]_0 - [A]) = 0 \qquad (3)$$

where

$$R = \frac{k'_1}{k_{-1}} = \frac{[A_0] - [A]_{eq}}{[A]_{eq}}$$

and $[A]_{eq}$ is the substrate A concentration at the equilibrium. From the results of the experimental runs in the presence of $HgCl_2$, the ratio R = 1.78 has been calculated. The use of experimental data collected during adsorption runs also allows to estimate the best value for k_{-1} constant (4.37×10^{-2} h^{-1}) through the application of an optimization procedure and the numerical integration of eq 3. The k'_1 value is 7.78×10^{-2} h^{-1}.

When the biodegradation is not inhibited by $HgCl_2$ addition, both the processes (a) and (b) have to be considered. In this case by using a pseudo-first-order kinetic law for the biodegradation the material balances become:

$$\frac{d[A]}{dt} = -k_{bio}[A] + k_{-1}[A_{ads}]Q - k'_1[A] \qquad (4)$$

$$\frac{d[A_{ads}]}{dt} = k_{-1}[A_{ads}]Q \qquad (5)$$

and can be integrated with the initial conditions $t = t_0$, $[A] = [A]_0$, and $[A_{ads}] = 0$. The adoption of a proper optimization procedure along with the data collected during the experimental runs of biodegradation allows to estimate the best value for the constant $k_{bio} = 4.43 \times 10^{-1}$ h^{-1}.

Toxicity Assessments. The standard methods for evaluating the potential effects of individual xenobiotics occurring in freshwater are based on a limited number of test organisms, which generally include at least one microalgal species, since it is well-known that algae represent one of the most vulnerable component of the ecosystems under stress (*34*). In a first approach, the toxicity of amoxicillin was tested on the unicellular Chlorophyte *P. subcapitata* (formerly *Selenastrum capricornutum*) according to standard methods (*35*, *36*). Amoxicillin concentrations tested ranged from 50 ng/L to 50 mg/L. The initial cellular density was 100 000 cells/mL, and the duration of tests was 96 h. However, in successive experiments the initial concentration of algal cells was reduced to 10 000 cells/mL to assess the influence of cellular density on the toxicity of amoxicillin. The results of the experiments have indicated that *P. subcapitata* is insensitive to amoxicillin within the range of test concentrations, irrespective of the size of the inoculum.

The regulatory practices recommended by official documents (*37*) suggest a tiered approach to the assessment of xenobiotic toxicity, the first level being represented by short-term bioassays to be carried out on a very sensitive species, such as *P. subcapitata*. If the EC_{50} of the compound divided by MEEC (maximal expected environmental concentration) is greater than or equal to 1000, no further testing should be performed. This would be the case of amoxicillin, but it is well-known that microalgae exhibit a wide degree of sensitivity to xenobiotics (*38*, *39*). In a following series of experiments, the toxicity tests have been extended to other microalgae, with the aim of evaluating the variability of species sensitivity to amoxicillin. The algal species selected included one member of Cyanophyta and Bacillariophyceae, which together with Chlorophyceae are the most representative algal taxa in freshwater ecosystems. On the basis of previous bioassays carried out on other pharmaceuticals (*40*), *S. leopoliensis* and *C. meneghiniana* were selected, respectively representative of Cyanophyta and Bacillariophyceae. In addition, amoxicillin was also tested on *C. ehrenbergii*, a very large unicellular green freshwater alga, frequently used to detect chemical water pollution (*30*). Amoxicillin was not toxic to eukaryotic algae but had a marked toxicity toward the blue-green alga *S. leopoliensis* (NOEC = 0.78 μg/L; LOEC = 1.56 μg/L; EC_{50} = 2.22 μg/L). The sensitivity of a Cyanophyta to antibiotics is not surprising; however, it is noteworthy that the values of NOEC are not far from those found in STP effluents. These data suggest that the use of a single species for assessing the toxic effects of a compound is not realistic. The adoption of a battery of microalgal strains belonging to major algal taxonomic groups should reduce the level of uncertainty of a risk assessment based on a single species (*41*). On the other hand, a too large number of bioassays could have costs and times of execution which do not lie within the range considered for toxicity tests in current regulations. In this paper the adoption of an intermediate

solution, by selecting for a preliminar toxicity assessment only three microalgae, respectively belonging to Cyanophyta, Bacillariophyceae and Chlorophyceae, is proposed. The results of this study show that *S. leopoliensis*, *C. meneghiniana*, and *P. subcapitata* could be suitable organisms for the evaluation of hazardous potential of xenobiotics.

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Supporting Information Available

HPLC analyses conditions and details about biodegradation experiments; graphic of half-life times for direct photolysis of amoxicillin in bidistilled water at pH 7.5, at varying season and latitude. This material is available free of charge via the Internet at http://pubs.acs.org.

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