

Electrochemical abatement of cefazolin: Towards a viable treatment for antibiotic-containing urine



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ABSTRACT

The release in the environment of antibiotics develop microbial resistance. In this work we investigated the feasibility of the electrochemical degradation in synthetic urine of cefazolin (CFZ), an antibiotic used for the treatment of antibiotic-resistant infections, which could be carried out in hospital wards on source-separated urine. CFZ is primarily excreted in urine without modifications. The removal of CFZ from human wastes would contribute to slow down the diffusion of CFZ-resistant bacterial strains more efficiently, compared to the treatment of much larger volumes of wastewater. CFZ can be electrochemically degraded using relatively low current density ($\geq 0.5 \text{ mA cm}^{-2}$) and without important generation of toxic by-products such as chlorate ($< 100 \mu\text{g L}^{-1}$). Oxidation of the S atoms of CFZ constitutes its main electrodegradation pathway, while the electrochemical generation of H_2O_2 accounts for most of the bacterial toxicity observed in electrodegraded mixtures. The treatment costs are not an issue because of the relatively low electricity consumption required for CFZ degradation (E_{EO} at 0.5 mA cm^{-2} current density was always $< 1 \text{ kWh m}^{-3}$), while the most challenging endeavours could be the manufacturing of a sufficiently compact apparatus and possibly its management in hospital wards.

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1. Introduction

The discovery of the first antibiotics between the end of the 1920s and the beginning of the 1940s marked the beginning of the antibiotic era, which we are still living in now (Zaffiri et al., 2012). Routine surgery and common infections are no longer life-threatening events, but rather occurrences with which we cope on a daily basis with relative safety. Antibiotics have indeed saved countless lives, with benefits from both economic and societal points of view (Wiles, 2015).

Nevertheless, bacteria and other microorganisms have biologically evolved following the intellectual evolution of their hosts, leading to antimicrobial resistance. This problem has emerged very early after antibiotic discovery (Cars, 2014), and until now it has been overcome with the continuous development and use of novel antibiotics, and with the combination of different chemotherapies

(e.g., the fortunate combination of clavulanic acid and β -lactam antibiotics; Zaffiri et al., 2012). Nevertheless, the co-evolution of resistant strains continues and is even accelerating, because of a series of combined effects: overuse of antibiotics, which can often be obtained without prescription; non-therapeutic use of these compounds (e.g., in cattle breeding; Cully, 2014); widespread use of antibacterial detergents and health-care products (Levy, 1998), and lack of understanding and awareness among the general public (McCullough et al., 2016).

Research on new antibiotics has not been very active in the latest 15–20 years, because these drugs have low profitability (Scannell et al., 2012). Furthermore, there is misuse of even the new antibiotics, many of which should just be used to treat infections resistant to common drugs only within hospitals. Alternatives to antibiotics are presently limited to bacteria-attacking viruses, i.e., bacteriophages, but their development is unlikely in the short-to mid-term because of intellectual-property protection problems and regulatory policy issues (Kingwell, 2015).

For these reasons, the perspective of a future without antibiotics is referred to as the “antibiotic apocalypse”. Such a claim is exceedingly gloomy because humanity survived $2 \cdot 10^5$ years without antibiotics (Coz and Worthington, 2017), but it is

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indisputable that the lack of viable antibiotics could cause huge societal and economic problems. The cumulative cost has been estimated as 100 trillion dollars worldwide by 2050, and the deaths due to antimicrobial resistance might pass from $7 \cdot 10^5 \text{ y}^{-1}$ today to $1 \cdot 10^7 \text{ y}^{-1}$ in 2050, overcoming even cancer-related mortality (WHO, 2014). Many strategies have been proposed, and some are already implemented to mitigate this problem. They are prioritization or even exclusivity of medical uses, renovated efforts in vaccination, increased attention to deficiencies in sanitation, and prescriptions tailored to more accurate diagnosis. Other likely requirements are the institution of a global surveillance on antimicrobial resistance and the decoupling between monetary reward and drug sale (Nathan and Cars, 2014).

When antibiotics must be administered, it should be considered that they are excreted to different extents in urine, often without modifications (Lienert et al., 2007). Once in urine, in the absence of an appropriate elimination procedure/technology, antibiotics may enter the environmental compartments via wastewater. Antibiotics can there promote microbial resistance (Bengtsson-Palme and Larsson, 2016), which can well occur at the sub-ppb concentrations commonly found in such compartments (Singh et al., 2019).

It is noticeable that urine represents only 1% of the total wastewater volume (Larsen and Gujer, 1996), but it is the primary contributor to the total antibiotics load (Landry and Boyer, 2016); indeed, antibiotic concentration in urine can exceed 1 g L^{-1} (Rein et al., 1973). The removal of antibiotics from source-separated urine is appealing, because it concerns small volumes of waste.

The use of direct and indirect electrochemical processes for the removal of organic pollutants has been previously investigated. A plethora of techniques has been proposed, such as anodic oxidation, (photo)electro-Fenton processes, sonoelectrochemistry, direct cathodic reduction, electrocoagulation, etc. The electrode material strongly influences the overall electrochemical process and affects both its selectivity and its efficiency: for instance, certain anodic materials promote selective substrate oxidation with high efficiency (i.e., chemical conversion), while others completely mineralize organic substrates to CO_2 and mineral ions (Comninellis, 1994). Several excellent reviews have been published regarding the use of electrochemical processes for the removal of recalcitrant pollutants and the role of the electrode material (Martínez-Huitle et al., 2015). Remarkable research efforts have also been directed to improve the performance of advanced oxidation processes for antibiotic removal, namely Fenton reactions, ozonation, sonochemistry, photocatalysis and ionizing radiations. For a

comprehensive review of the works related to antibiotics degradation through advanced oxidation processes, please refer to the exhaustive works by J. Wang and coworkers (Wang et al., 2019).

Compared to other advanced oxidation processes, the electrochemical treatment shows several potential advantages for the removal of antibiotics from urine, because no addition of chemicals is required and process automation is relatively easy, which would be important for urine treatment in hospitals. Electrode materials have already been developed in industrial processes such as water oxidation and chloralkali plants (Karlsson and Cornell, 2016). Energy demand is usually affordable, and it is easy to estimate and control (Radjenovic and Sedlak, 2015). Electrochemical treatments need electricity-conductive matrices (Barazesh et al., 2016), and urine satisfies this requirement because of its ionic content (Marangella et al., 1985). In contrast, other advanced oxidation processes that are able to efficiently oxidize antibiotics in light matrixes (e.g., in fresh water) might be more affected by the presence of high ionic concentration.

In this work, we studied for the first time the electrochemical abatement of the antibiotic cefazolin (CFZ) on commercial platinum electrodes in synthetic urine matrix, focusing on:

- the effects of pH;
- the influence of the different components of the urine matrix;
- the possible formation of by-products such as chlorate (Radjenovic and Sedlak, 2015);
- the energy efficiency and the cost figures, which are of crucial importance in the perspective of the implementation of treatment facilities for antibiotic-containing urine in hospitals.

A major reason for the choice of CFZ as substrate for this study is that it is primarily excreted in urine without modifications (Rein et al., 1973). CFZ is potentially very vulnerable to the problem of antibiotic resistance.

2. Experimental

2.1. Chemicals

Cefazolin, creatinine, sodium citrate dihydrate, Na_2SO_4 , NaCl , $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, NH_4Cl , NaClO_4 , KCl and K_2CO_3 were purchased from Sigma-Adrich; KNO_3 and potassium oxalate were from Merck; KH_2PO_4 , K_2HPO_4 , acetonitrile (gradient grade) and urea from Carlo Erba. All chemicals were of at least analytical grade.

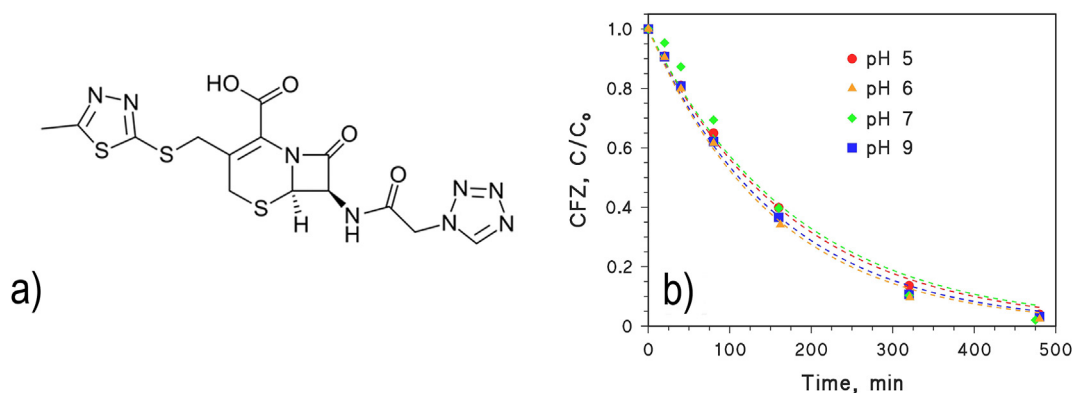


Fig. 1. (a) Structural formula of CFZ, (b) concentration vs. time profiles for the galvanostatic electrochemical degradation of $100 \mu\text{M}$ CFZ with $I = 0.5 \text{ mA cm}^{-2}$ at different pH values, in the presence of 10 mM PB . The $100 \mu\text{M}$ initial concentration of CFZ was selected as default, because it is experimentally amenable, while it represents the lower range of typical urine levels.

2.2. Synthetic urine

The composition of the synthetic urine is shown in Table S1 of the Supplementary Material (hereinafter SM), and it is the same as reported by Giannakis et al. (2017). Reagents were dissolved in Milli-Q water and two stock solutions (five times more concentrated than shown in the table) were prepared. In solution A we dissolved NaCl, CaCl₂, KCl, NH₄Cl, creatinine and urea, while solution B contained Na₂SO₄ and KH₂PO₄. These solutions were diluted and mixed with the stock cefazolin solution right before the experiments, to avoid precipitation of calcium phosphates (Chow, 2001) and CaSO₄ ($K_s = 4.93 \times 10^{-5}$, anhydrous; $K_s = 3.14 \times 10^{-5}$, dihydrate) (Linde, 2002) in a concentrated stock solution containing all the reactants.

2.3. Electrochemical cefazolin abatement and analytical methods

The electrochemical experiments were carried out with a standard set-up composed of a computer-controlled potentiostat/galvanostat (AUTOLAB PGSTAT12). The cell was an undivided three-electrode cell. The working, counter and reference electrodes were a Pt sheet (1 cm²), a glassy carbon rod (2 mm diameter, 75 mm length) and a Ag/AgCl/KCl (3 M) electrode. The degradation experiments were carried out with 20.0 mL of CFZ-spiked electrolyte under magnetic stirring, and always under galvanostatic control (i.e., by keeping constant the current density by suitably modifying the working electrode (WE) potential). Current density was in the range of 0.5–150 mA cm⁻². During the galvanostatic electrochemical experiments, the WE potential was continuously recorded to get insight into the possible electron-transfer processes operating at the WE during the whole experiment. Sequential withdrawals of electrolyte aliquots were carried out at scheduled times, and the samples were analyzed by liquid chromatography (HPLC-UV, LC-MS), ion chromatography, UV-vis spectroscopy for H₂O₂ determination, and with an analyzer of total organic carbon and total nitrogen (TOC and TN). Furthermore, toxicity was evaluated with a bioluminescence inhibition assay carried out by using the marine luminescent bacterium *Vibrio fischeri* (Microtox®). The methodological details are reported in the SM.

3. Results and discussion

3.1. The role of pH in cefazolin hydrolysis and electrochemical abatement

Cefazolin (CFZ, Fig. 1a) is known to undergo hydrolysis with pseudo first-order rate constant $>0.1 \text{ h}^{-1}$ at 35 °C, under both acidic (<2) and basic (>10) pH values (Yamana and Tsuji, 1976). CFZ exhibits a hydrolysis-rate minimum at pH 6 (Rattie et al., 1979) and, at physiological pH, it has $t_{1/2} \approx 14$ days. This feature poses serious concern about the possibility for CFZ-resistant bacteria to develop in natural waters, because CFZ is potentially persistent and could occur at sufficiently high concentration. Actually, CFZ biodegradation is understandably slow, while the typical phototransformation lifetimes are in the order of some weeks to 1 month (Fabbri et al., 2015). Such lifetimes could not be short enough to ensure sufficient CFZ photodegradation in river water, under normal flow conditions (Vione et al., 2018).

During the electrochemical experiments we always observed that CFZ degradation followed first-order kinetics ($C_t/C_0 = \exp(-kt)$), where C_0 and C_t are the initial CFZ concentration and that observed after t minutes of electrochemical treatment, while k is the first-order kinetic constant). The initial degradation rate of CFZ in the process is the product between k and the initial concentration ($\text{rate} = k C_0$).

Around neutrality (pH 5–9), where the molecule is stable (see Fig. S1 in SM, for the hydrolysis kinetics at pH 6), the pH value has insignificant effect on the electrochemical degradation of CFZ ($C_0 = 100 \text{ }\mu\text{M}$). Indeed, we observed similar rate constants (variations $\approx \pm 7\%$) for degradation experiments carried out at pH 5, 6, 7, and 9 in 10 mM phosphate buffer (hereinafter, PB) under galvanostatic conditions with a current density $I = 0.5 \text{ mA cm}^{-2}$ (Fig. 1b). In these conditions, $\sim 95\%$ CFZ abatement could be obtained after 8 h, thereby consuming a total electrical charge of 14.4 C (computed as $Q = I \times t \times A$, where I is the current density, i.e., 0.5 mA cm^{-2} , t the duration of the electrochemical process, and $A = 1 \text{ cm}^2$ the geometric area of the WE). Considering the volume treated (20 mL) and the cell potential (Table S2) it is possible to estimate the electrical energy per order (E_{EO} , expressed in kWh m⁻³), which represents the energy needed to decrease by one order of magnitude the concentration of the target molecule in a 1 m³ solution, according to the following equation:

$$E_{EO} = \frac{Eit}{V \log \frac{C_0}{C}} \quad (1)$$

where E is the cell potential (reported as column 5 in Table S2), i is the current (see column 2 in Table S2, which reports the current density I that gives i when multiplied by the electrode surface of 1 cm²), V is the volume of water treated in the time t , and C_0 and C are the initial and final CFZ concentrations (Radjenovic and Sedlak, 2015).

The values of E_{EO} , CFZ degradation rate, WE potential (average of the WE potential during the electrochemical experiments), current density used, cell potential (difference between the working and counter electrode potential, measured with a multimeter at the end of the process), and the faradaic efficiency of each electrochemical degradation experiment are reported in Table S2.

The faradaic efficiency (FE) is defined as the ratio between the charge contributing to the wanted reaction(s) (i.e., antibiotic oxidation) and the total charge consumed. FE was here calculated as the ratio between the moles of electrons employed to oxidize CFZ and the total moles of electrons supplied during the electrochemical process. The moles of electrons that effectively oxidize CFZ were inferred from the moles of converted CFZ, multiplied by the number of electrons needed for the conversion of one CFZ molecule. FE was calculated as follows:

$$FE = \frac{(\text{mol CFZ converted})n}{\text{total mol electrons}} = \frac{(\text{mol CFZ converted})nF}{it} \quad (2)$$

Where n is the average number of electrons abstracted from a CFZ molecule during the process, i is the current, t the time, and F is the Faraday constant ($9.65 \times 10^4 \text{ C mol}^{-1}$). The most conservative estimate of FE is made assuming $n = 2$, because at least two electrons must be abstracted from CFZ to obtain a stable product (Table S2 of the SM). If the oxidation of CFZ goes beyond the first two-electron oxidation step, the actual FE value will be underestimated by Eq. (2) and the corresponding FE data reported in Table S2 should be regarded as lower limits (*vide infra*).

The values of E_{EO} varied from 0.5 kWh m^{-3} at pH 9 to 0.8 kWh m^{-3} at pH 7. They were quite stable, or even decreased during the electrochemical treatment (see Fig. S2–S5 in SM). In the latter case, we considered the initial E_{EO} values.

3.2. Effect of the progressive addition of urine components: phosphate, chloride, and sulfate

Urine is a complex matrix containing both inorganic salts and organic compounds. Marangella et al. (1985) have studied urine

composition in healthy subjects and stone-developers, and the composition for the former is reported in Table S3. Urine composition varies depending on the diet, and there are significant variations in the excreted quantities of Ca, K, Mg, Na, oxalate and citrate (Siener and Hesse, 2002). Alkali and alkaline earth cations have high oxidation states and low reduction potentials: they are mainly spectators of the redox processes. In contrast, the anions can be electrochemically oxidized. Anion oxidation could compete with the degradation of CFZ, but it could also trigger indirect degradation mechanisms through the formation of redox mediators (e.g., $\text{SO}_4^{\cdot-}$ and $\text{Cl}_2^{\cdot-}$). Indirect oxidation may enhance degradation (Radjenovic and Sedlak, 2015), but it can also give rise to side products with toxic properties. This is especially true for chloride that can be oxidized to chlorite, chlorate and perchlorate, with well-known toxicity (Jung et al., 2010).

We studied the effects of the urine anions phosphate, sulfate and chloride by progressively adding these components to the studied system, keeping pH at 6 with a phosphate buffer (Fig. 2). By increasing the phosphate concentration from 10 to 20 mM, the degradation rate increased by 25%. We ruled out the possibility that the increase in the degradation rate was only due to an ionic strength increase, by carrying out the electrochemical treatment in the presence of 162 mM NaClO_4 . In this case, the degradation rate was significantly lower compared to 20 mM phosphate (Table S2). Possibly, phosphate may improve CFZ degradation via the formation of peroxydiphosphate. The ability of oxidizable anions to act as electron shuttles in electrodegradation processes has already been reported (Radjenovic and Sedlak, 2015). Peroxydiphosphate has a standard reduction potential of 1.86 V vs. Ag/AgCl (i.e., 2.07 V vs. NHE (Mesaros et al., 2003)), which is included in the WE potential range explored in the present work (1.73–1.91 V vs. Ag/AgCl, see Table S2). The electrochemical oxidation of PO_4^{3-} to $\text{P}_2\text{O}_8^{2-}$ is thermodynamically possible.

When Cl^- was added to the electrolyte containing 10 mM phosphate at pH 7, an insignificant effect was observed for $[\text{Cl}^-] = 10$ mM. Enhancement of CFZ degradation occurred with $[\text{Cl}^-] = 100$ mM (Fig. 3). The effect of added chloride seems to be relatively small as compared to phosphate, for which acceleration could already be observed by increasing the phosphate

concentration from 10 to 20 mM. This finding is also coherent with the comparable degradation profiles observed with 20 mM phosphate, and with 20 mM phosphate upon addition of 140 mM chloride (Fig. 2). The chloride effect could be due to an indirect oxidation of CFZ. Indeed, the oxidation of Cl^- to Cl_2 requires a potential of 1.36 V vs. Ag/AgCl (Karlsson and Cornell, 2016), to be compared with the experimentally measured working electrode potential of 1.76 V vs. Ag/AgCl. The mediating role of chloride ions during anodic transformation has been reported by Comninellis and Nerini (1995), and it has been investigated in-depth by the De Battisti's group (e.g., Bonfatti et al., 2000). They proposed an extension of the reaction scheme originally suggested by Comninellis (1994), where oxychloro species that are able to oxidize the organic substrate(s) act as intermediates in chlorine evolution reactions. In this framework, the electrochemical transformation of polyaromatic compounds on platinum is mainly due to the electrogeneration of active chlorine at the electrode surface (Panizza and Cerisola, 2003).

During the transformation of CFZ, chloride oxidation could trigger the production of further species such as toxic chlorite, chlorate and perchlorate. In the relatively mild oxidation conditions applied here (with $i = 0.5 \text{ mA cm}^{-2}$, the applied potential at the WE was in the 1.72–1.93 V range) such processes should be negligible (*vide infra* for further discussion).

The addition of 15 mM sulfate to the systems containing 20 mM phosphate, or 20 mM phosphate + 140 mM chloride, had limited effects on the CFZ degradation rate constant (Table S2). Differences in the rate constant values observed in the three systems were within $\pm 15\%$, which is of the same order of magnitude as the experimental uncertainty.

3.3. Effect of urea, ammonium, and organics

Urea is the most concentrated species in urine ($\approx 0.3 \text{ M}$), and its anodic degradation has been studied in detail in the literature (Boggs et al., 2009). The electrochemical degradation of urea opens up the possibility of a competition with CFZ. We studied CFZ electrodegradation at pH 6, in the presence of 0.33 M urea and 20 mM PB. In an additional degradation experiment, 140 mM Cl^- was also added. The observed degradation rates matched very well the results obtained with 20 mM PB only (Table S2, Fig. S6a/b),

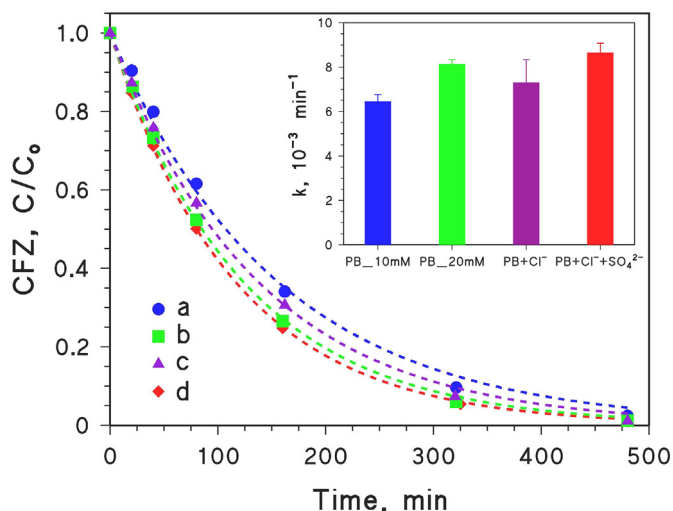


Fig. 2. Time trends of CFZ concentration (C_0 100 μM) in the case of the electrochemical degradation of different matrices with $i = 0.5 \text{ mA cm}^{-2}$ at pH 6: (a) 10 mM PB; (b) 20 mM PB; (c) 20 mM PB + 140 mM Cl^- ; (d) 20 mM PB + 140 mM Cl^- + 15 mM SO_4^{2-} . The figure insert shows the values of the first-order degradation rate constants k of CFZ in the different experiments. The error bounds represent the 95% confidence limits.

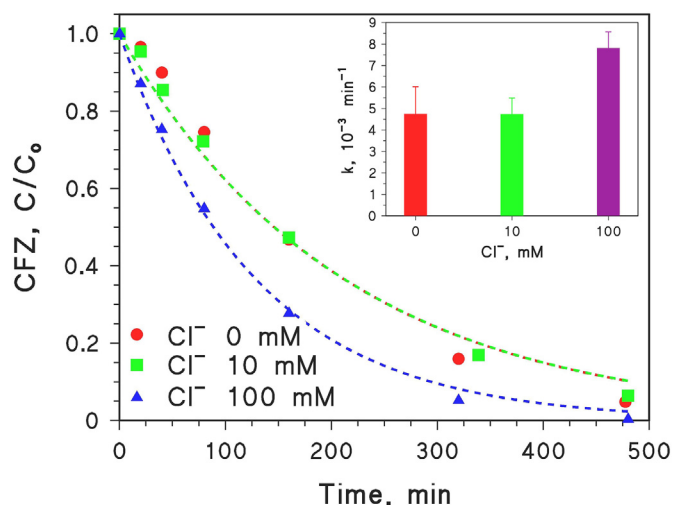


Fig. 3. Concentration vs. time profiles for the electrochemical degradation of 100 μM CFZ with $i = 0.5 \text{ mA cm}^{-2}$ at pH 7, in the presence of 10 mM phosphate with different Cl^- concentrations. Inset: values of the first-order degradation rate constants k in the different experiments. The error bounds represent the 95% confidence limits.

thereby suggesting that urea has very limited effect on the electrochemical degradation of CFZ.

Another set of experiments considered the effect of organic acids such as citric and oxalic, which are usually found in urine in the μM to mM range (see Table S3 in SM) (Marangella et al., 1985). We tested the effects of 0.25 mM oxalic acid and 3 mM citric acid, separately and in combination, in the presence of 20 mM PB and 140 mM chloride at pH 6. The observed inhibition effect (25–35%, Table S2 and Fig. S6c) can be almost exclusively ascribed to oxalic acid, although it was six times less concentrated than citric acid. Indeed, citric acid alone did not affect CFZ degradation significantly. The different behaviour of these two acids is not easy to rationalize, because both of them can be oxidized on Pt electrodes (for oxalate: Anson and Shultz, 1963; for citrate: Chen et al., 2012).

Creatinine increased the degradation rate of CFZ slightly (0.93 min^{-1} , compared to 0.81 min^{-1} with 20 mM PB alone), but such an increase (<15%) was within the experimental repeatability. The presence of NH_4^+ , added in the form of ammonium nitrate to avoid the simultaneous presence of chloride that could interfere with the degradation kinetics, also caused a slight degradation increase ($\approx 20\%$) compared to 20 mM PB alone (Fig. S6b).

3.4. CFZ degradation in synthetic urine

The next step was the study of CFZ degradation in synthetic urine. The degradation of $100 \mu\text{M}$ CFZ in such a matrix was very similar to that observed with 20 mM phosphate alone (Fig. S6a). We observed that the effects on CFZ degradation of the separate components of synthetic urine were generally marginal (the degradation rates were usually affected by less than 10–20%). Competing effects of slight acceleration and slight inhibition would cancel out. In synthetic urine, CFZ was degraded at pH 6 and 1.78 V vs. Ag/AgCl (WE current density of 0.5 mA cm^{-2}), yielding $E_{\text{EO}} = 0.32 \text{ kWh m}^{-3}$. The faradaic efficiency was 9.2% when assuming two-electron oxidation. These figures are very close to those obtained at pH 6 in the presence of phosphate and chloride only, thereby suggesting that the degradation of CFZ in urine could occur without significant losses in terms of rate, energy and faradaic efficiency, compared to a standard electrolyte.

3.5. Effect of CFZ concentration

The CFZ concentration in serum after 500–1000 mg antibiotic treatment is in the order of $100 \mu\text{M}$ (Rattie and Ravin, 1975). The CFZ concentration in urine has been reported to even exceed 2 mM in patients with normal renal function, within the first 6 h from treatment (Rein et al., 1973). For this reason, we also tested the abatement of CFZ by increasing its initial concentration by 10-fold ($C_0 = 1 \text{ mM}$) (Fig. 4). We observed a higher degradation rate comparing to the experiments at $100 \mu\text{M}$ CFZ, but also a less effective degradation after 8 h treatment (9% residual CFZ, vs. 3% in the $100 \mu\text{M}$ experiment). In other words, by increasing the initial CFZ concentration one decreases the degradation rate constant (k) while increasing the degradation rate ($k C_0$).

The higher initial concentration nearly doubled E_{EO} , showing that the electrocatalytic CFZ degradation was not a perfect first-order reaction in our experimental configuration (Bolton et al., 2001). Indeed, one needs more energy to degrade nine tenths of 1 mM CFZ (i.e., $900 \mu\text{M}$) than to degrade the same percentage of $100 \mu\text{M}$ CFZ (i.e., $90 \mu\text{M}$), in agreement with previous reports (Lanzarini-Lopes et al., 2017). E_{EO} only doubles to allow for the degradation of a CFZ amount that is ten times larger, one has a 5-fold increase in the faradaic efficiency (ranging from 9% for $100 \mu\text{M}$ CFZ, to more than 50% for 1 mM CFZ). At larger CFZ concentration the process is energetically more efficient, but it requires

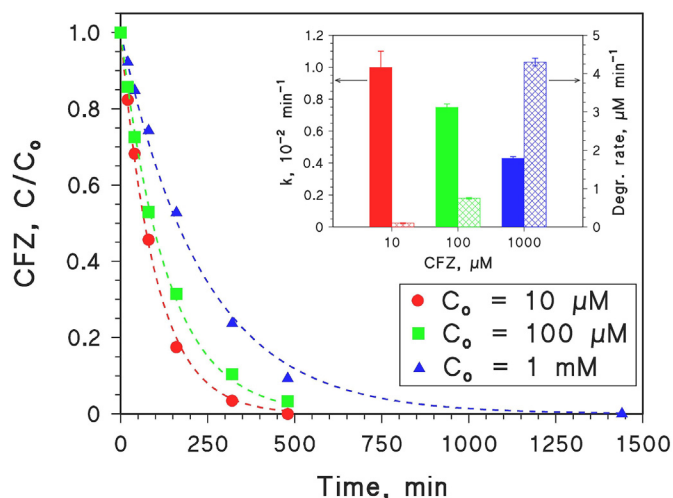


Fig. 4. Time trends for the electrochemical degradation of 10 μM , 100 μM and 1 mM CFZ with $I = 0.5 \text{ mA cm}^{-2}$ in artificial urine. Inset: values of the first-order degradation rate constants k of CFZ (solid bars) and of its initial degradation rates (products of k times the initial CFZ concentration, cross-dashed bars). The error bounds represent the 95% confidence limits.

more time to attain the same degradation percentage.

Antibiotic resistance is developed at very low antibiotic concentrations, and for CFZ this limit has been estimated at 2 nM (Bengtsson-Palme and Larsson, 2016). For this reason, we also carried out degradation starting from $C_0 = 10 \mu\text{M}$. Symmetrically to the above-mentioned considerations, in this case the abatement after 8 h was more pronounced (with <1% residual CFZ), and there was a decrease in both E_{EO} and faradaic efficiency. Residual CFZ was above 2 nM, but if treated urine is then discharged into wastewater the final resulting concentration (due to a dilution by two orders of magnitude: Landry and Boyer, 2016) would fall below the antibiotic resistance threshold. These data demonstrate that the electrochemical treatment can effectively remove CFZ at initial concentrations that are relevant to real-world applications, to reach levels that would fall below the resistance threshold in wastewater.

3.6. Effect of applied current, considerations on efficiency and costs

Intuitively, the degradation of CFZ became faster with increasing current density, as shown in Fig. 5. We observed this effect both in the presence of 10 mM PB at pH 7 (Fig. 5a), and in synthetic urine (Fig. 5b). The advantage of a higher current density is the shorter time needed to reach the desired degradation, which in turn reduces the requirements in terms of reactor size and allows for the treatment of larger volumes. Energy requirements increase with increasing current density and, coherently, the E_{EO} value increased from 0.32 kWh m^{-3} at 0.5 mA cm^{-2} to 3.77 kWh m^{-3} at 5 mA cm^{-2} , and up to $\approx 16 \text{ kWh m}^{-3}$ at both 50 and 150 mA cm^{-2} (Table S2).

At larger current density the WE potential becomes more oxidizing, which increases the possibility of side reactions such as chlorate production. Indeed, after 20 min of electrolysis at $I = 150 \text{ mA cm}^{-2}$ we achieved very good CFZ degradation (the residual antibiotic was <0.5% C_0), but we recorded $[\text{ClO}_3^-] = 580 \mu\text{g L}^{-1}$ with 0.44% faradaic efficiency for ClO_3^- production. By comparison, the chlorate regulatory limit set by WHO is $700 \mu\text{g L}^{-1}$ (Liu et al., 2017). In all other cases we had $[\text{ClO}_3^-] < 100 \mu\text{g L}^{-1}$ (the limit of detection of our experimental setup). A larger current density requires more careful control of all the operational parameters (residence time, reactor volume, cell potential, etc.) to minimize chlorate production.

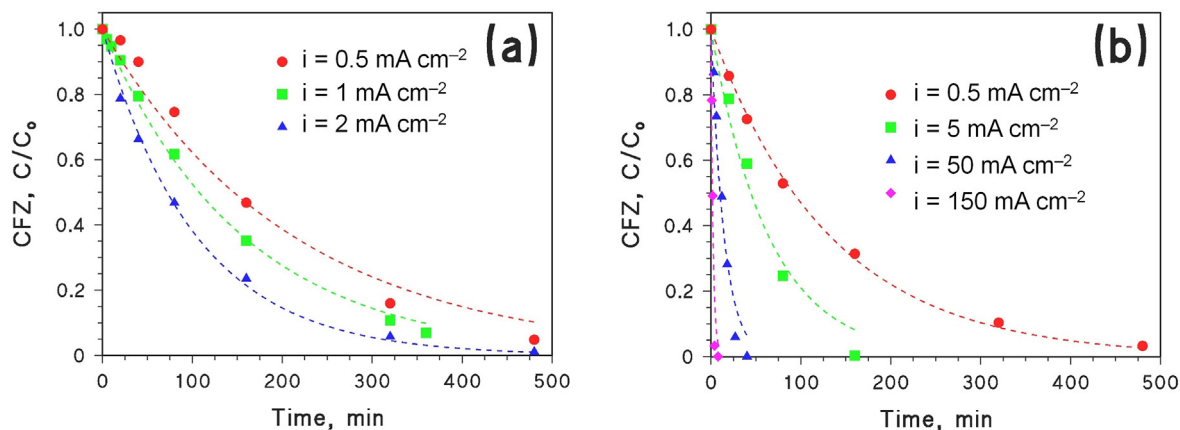


Fig. 5. Time trends for the electrochemical degradation of 100 μM CFZ with different applied currents (note that, for $i = 0.5$ mA, the current density is $i = 0.5$ mA cm $^{-2}$ because the electrode surface is 1 cm ^{2} , and so on), in (a) 10 mM PB at pH 7, and (b) artificial urine.

2 nM is the threshold CFZ concentration, below which one has to go to avoid the development of bacterial resistance, it is possible to roughly assess the electrical energy costs that are required to degrade CFZ in urine down to that level. By considering a rather elevated initial CFZ concentration of 1 mM (which requires an abatement of ~ 6 orders of magnitude to go below 2 nM), and neglecting the fact that treated urine will eventually be diluted in a much larger volume, and considering as well $E_{\text{EO}} = 0.61$ kWh m $^{-3}$ (this is also precautionary, because E_{EO} decreases with concentration as shown before), we obtain that the electrochemical treatment will require at most $0.61 \times 6 \approx 3.7$ kWh m $^{-3}$. This corresponds to a treatment cost of less than 1.2 \$ m $^{-3}$ by assuming an average electricity cost of 0.32 \$ kWh $^{-1}$ (Faruqui and Sergici, 2010). In most countries, treatment costs would not exceed 0.5 \$ m $^{-3}$.

By comparison, the current cost of wastewater treatment in Italy is 0.38 \$ m $^{-3}$, after which one obtains wastewater that can be discharged into the environment but without significant degradation of the antibiotics (Behera et al., 2011). CFZ is mainly found in urine, which makes up no more than 1% of the wastewater volume. One has approximately 10 L urine for every 1 m ^{3} wastewater. The electrochemical treatment of 10 L urine would cost ~ 0.01 \$, which means that in the end the treatment of wastewater would cost 0.38 \$ m $^{-3}$ without CFZ degradation, or 0.39 \$ m $^{-3}$ with CFZ degradation (of course, when considering electricity as the only cost unit). Much more than the cost of electricity, the real challenge is actually to separately treat urine before it is discharged into the overall wastewater. At the moment such a goal might be achieved in hospital wards, and be targeted at the antibiotics reserved to hospital settings. The latter are usually employed to treat infections caused by bacteria that are resistant to other antibiotics, and understandably the development of antibiotic resistance would be extremely problematic for these last-resort drugs. In such a context, electrochemical degradation could be interesting because of its effectiveness and reasonable cost (Boyer and Saetta, 2019).

3.7. Sulfate formation curves, and considerations about faradaic efficiency

CFZ contains three sulfur atoms (Fig. 1a), and sulfate could be released into the solution during degradation. When operating with a sulfate-free electrolyte, we were able to follow sulfate formation from CFZ as shown in Fig. 6. Depending on the experimental conditions, sulfate concentration reached 70–140 μM after 8 h of electrolysis at 0.5 mA cm $^{-2}$ current density (total charge employed, 14.4 C). Stoichiometric considerations, made on the basis of CFZ

initial concentration and the sulfate formation levels, suggest that a sulfate ion was released on average for every CFZ molecule degraded. This finding allows for a better estimate of the faradaic efficiency of the process. During electrochemical degradation, S is transformed from the CFZ sulfide (R-S-R', oxidation number of zero) to sulfate, where the S oxidation number is 6. The formation of a sulfate ion requires 6 electrons, which means that the faradaic efficiency figures listed in Table S2 (obtained by considering two-electron oxidation) are conservative estimates. In many cases those figures should be multiplied by at least 3, which makes faradaic efficiency reach levels of 25–30%. An exception to this scenario is certainly represented by the electrolysis of 1 mM CFZ, where the estimated faradaic efficiency was already $>50\%$ by considering two-electron oxidation. In this case, the elevated concentration of CFZ would prevent its full oxidation to sulfate, at least at the time scales of our experiments.

While S underwent partial mineralization, the same did not happen to carbon and nitrogen. The time trends of both TOC and TN during CFZ electrodegradation for up to 50 h (i.e., 3000 min, Fig. S7 in SM) indicated that, within experimental errors, carbon and nitrogen were not mineralized. The only doubt could concern the N atoms occurring in the two 5-member rings that may escape detection by being converted into N $_2$ instead of NO, during TN measurements based on catalytic combustion (Minella et al., 2016).

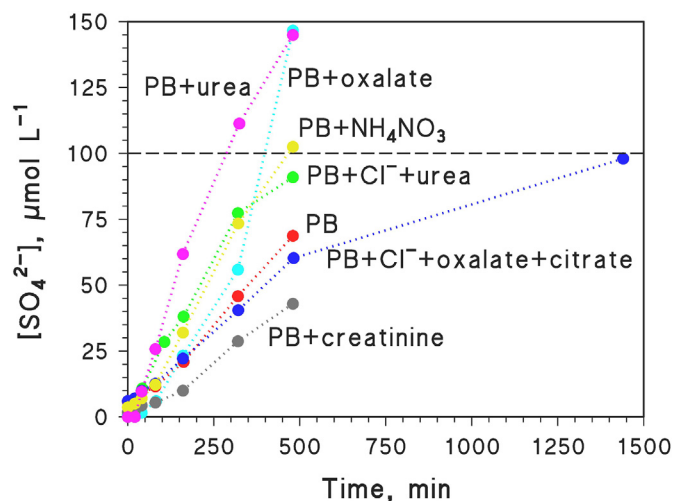


Fig. 6. Concentration vs. time profiles for the electrochemical formation of sulfate from 100 μM CFZ in different matrices. The dashed horizontal line shows the sulfate level caused by the release of one of the three S atoms of CFZ.

3.8. Bacterial toxicity trend during treatment, and identification of transformation intermediates

The assessment of the time evolution of bacterial toxicity may give some insight into the antibiotic activity of the CFZ transformation intermediates. The toxicity of CFZ to *Vibrio fischeri* bacteria was tested in phosphate buffer at pH 7, that is, under conditions that are well known to cause no additional stress to the test organisms, except for the presence of the test compound. The results (see Fig. S8 in SM) show that CFZ has $EC_{50} = 7.4 \text{ mmol L}^{-1}$, which is a rather high value. The likely reason is that CFZ is mostly active against Gram + bacteria, and much less towards Gram- ones such as *V. fischeri*. The bacterial toxicity of CFZ in the test solutions could still be measured with due confidence.

The main problem found with bacterial toxicity measurements in synthetic urine is that conditions of pH 6 are exceedingly toxic for *V. Fischeri*, while at pH 7 the matrix was not stable and precipitation reactions occurred. Most toxicity measurements were carried out at pH 7 by PB only, while synthetic urine was only used for comparison purposes. Some toxicity (10% bacterial inhibition, due to the parent compound) was already observed in the presence of $100 \mu\text{mol L}^{-1}$ CFZ in PB at pH 7. Toxicity increased significantly during the electrochemical treatment (see Fig. 7) and it would mostly accounted for by the formation of H_2O_2 (reaching $\sim 10^{-4} \text{ mol L}^{-1}$, see Fig. S9 in SM). The contribution of H_2O_2 to bacterial toxicity is shown in Fig. 7, which suggests that CFZ transformation intermediates would play little role.

To assess the bacterial toxicity attributed to H_2O_2 , the concentration of the latter was quantified as a function of treatment time, and toxicity was then measured for solutions (PB, pH 7, no CFZ) containing the same concentration of H_2O_2 as the electrochemically degraded samples. It can be seen in Fig. 7 that H_2O_2 accounted for virtually all bacterial toxicity increase up to ~ 250 min treatment time, and for the majority of the toxic effect afterwards. Very similar effects were seen for CFZ degradation in both PB and synthetic urine (see Fig. S9 in SM). These findings suggest that the electrodegraded mixtures did not show a large increase of the antibiotic activity by the transformation intermediates, compared to the initial CFZ. They do not exclude that the intermediates might still give rise to antibiotoxic resistance, and further work will be needed on this subject.

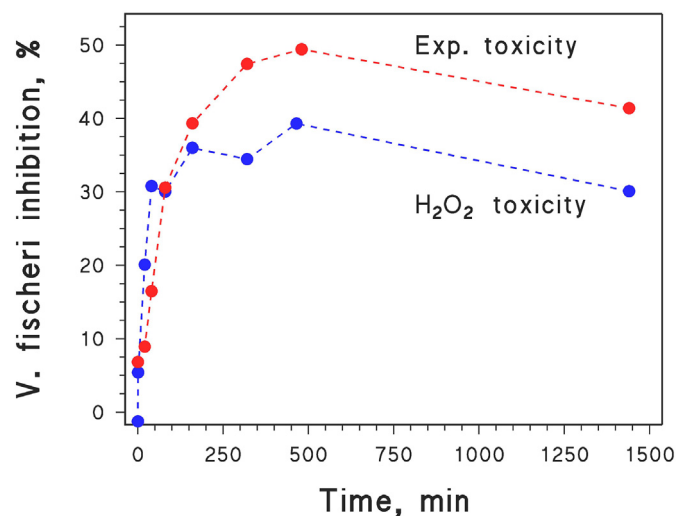


Fig. 7. Time trend of *Vibrio fischeri* inhibition (Microtox® test) upon electrochemical degradation of $100 \mu\text{mol L}^{-1}$ CFZ (PB, pH 7, $I = 0.5 \text{ mA cm}^{-2}$), and comparison with inhibition accounted for by electrogenerated H_2O_2 .

The five CFZ transformation intermediates were identified by LC-MS, they had shorter retention times than CFZ, and their proposed structures are reported in Table 1. The MS spectrum of CFZ showed several peculiar losses previously reported (Heller and Ngoh, 1998). The two transformation intermediates with m/z 471 can be assigned as mono-oxidized derivatives ($\text{S} \rightarrow \text{S}=\text{O}$) on the basis of MS fragmentation, and in agreement with literature findings (Li et al., 2013). When taking into account the relatively low reactivity of the S atom of the thiadiazole ring, we can hypothesize that oxidation involved the other two S atoms occurring in the CFZ molecule. The fragmentation pattern of the intermediate 471B shows a product ion at m/z 339, which makes a close analogy with CFZ and is consistent with the oxidation of the thioether (m/z 323) arising from the detachment of 2-mercapto-5-methyl-1,3,4-thiadiazole. This datum agrees with the proposed structure, where the sulfur atom close to the β -lactam structure is oxidized. The m/z 321 and m/z 293 fragments can be explained by a subsequent loss of H_2O and CO_2 from m/z 323.

The different MS² spectrum of 471A compared to 471B allows for the exclusion of thioether oxidation in the latter case. The product ion at m/z 372 matches with 5-methyl-1,3,4-thiadiazole radical elimination. One observes subsequent losses of 29 Da (loss of HCO radical) and 28 Da (CO or N_2 loss) to give the signals at m/z 343 and 315. The product ion m/z 277 can be attributed to the combined detachment of the mercaptothiadiazole moiety and a carboxylic group. The preferential formation of the 471A and 471B isomers was also demonstrated by Li et al. (2016) during the process of CFZ removal with potassium permanganate.

The other intermediates had m/z values lower than CFZ. For instance, the compound with 6.25 min retention time had m/z 409. It shares the product ion m/z 277 with the 471A intermediate (loss of 2-mercapto-5-methyl-1,3,4-thiadiazole), which suggests that it might be formed upon detachment of a carboxylic group from CFZ. The product ions at lower m/z values (249, 221) can be justified with the subsequent loss of CO or N_2 .

Two structural isomers at m/z 443 were detected, and attributed to dioxidized and decarboxylated CFZ derivatives. For 443A, in analogy with 471A, the 5-methyl-1,3,4-thiadiazole loss to give the product ion at m/z 343 suggests us that the oxidation process did not involve the part of the molecule that was detached. The signal at m/z 171 is consistent with the oxidized form of the product ion m/z 156 observed with CFZ, which confirms the involvement in oxidation of a sulfur atom close to the β -lactam structure. Unfortunately, the 443B isomer did not afford structurally informative losses. One might also hypothesize a second structure where one of the two sulfoxide groups does not occur on the aminocephalosporanic moiety.

4. Conclusions

In the present work the feasibility of the electrochemical abatement of CFZ in urine was demonstrated. pH has little influence on CFZ degradation and the process is accelerated by the ions phosphate and chloride. The organic compounds typically occurring in urine show negligible to weak inhibition of CFZ degradation.

CFZ can be degraded at current densities ranging from 0.5 to 150 mA cm^{-2} . Chlorate formation (although below the 700 ppb regulatory limit) is only observed with the largest current density, and when CFZ has already disappeared. The FE of the process is directly proportional to CFZ concentration and inversely proportional to current density, exceeding 50% with 1 mM CFZ at 0.5 mA cm^{-2} .

The energy efficiency per order at low current density (0.5 mA cm^{-2}) is 0.32 kWh m^{-3} , which is promising for the implementation of treatment facilities for antibiotic-containing

Table 1Transformation intermediates detected and identified by LC-MS, upon electrodegradation of 1 mmol L⁻¹ CFZ with PB at pH 7 (0.5 mA cm⁻² current density).

Compound structure	m/z	tr (min)	MS ² Fragment, m/z (% relative abundance)
	455	6.9	CFZ : 323 (60), 295 (50), 156 (100).
	471 A	1.72	372 (15), 343 (30), 315 (30), 277 (30)
	471 B	3.36	339 (20), 321 (100), 293 (40)
	443 A	1.62	415 (10), 343 (10), 315 (100), 272 (50), 231 (50), 171 (100)
	443 B	5.98	—
	409	6.25	277 (100), 249 (80), 221 (100)

urine in hospitals.

The CFZ transformation intermediates arise from the oxidation of the different CFZ sulfur atoms ($S \rightarrow S=O$, plus eventual formation of SO_4^{2-}). The intermediates do not show important bacterial toxicity, which increases as a function of treatment time but is largely accounted for by electrogenerated H_2O_2 .

The CFZ electrochemical degradation was carried out on a commercial Pt electrode. The electrode material can affect abruptly the selectivity and overall efficiency of the process, future works will investigate the relationship between electrode material and CFZ electrodegradation performance in urine-matrix and the feasibility of the electrochemical degradation of CFZ in real urine sample and/or containing other antibiotics.

CRedit authorship contribution statement

Fabrizio Sordello: Data curation, Formal analysis, Methodology, Writing - original draft. **Debora Fabbri**: Methodology, Investigation, Data curation, Validation. **Lorenzo Rapa**: Investigation, Data curation, Writing - original draft. **Claudio Minero**: Conceptualization. **Marco Minella**: Data curation, Supervision, Writing - review & editing. **Davide Vione**: Conceptualization, Funding acquisition, Supervision, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jclepro.2020.125722>.

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