Testing a novel MMO-Ti/RuO₂IrO₂ electrode to reduce the dangerousness of antibiotic polluted urines by Fenton-based processes

Isabelle M.D. Gonzaga^{a,b,c}, Angela Moratalla^c, Katlin I. B. Eguiluz^{a,b}, Giancarlo R. Salazar-Banda^{a,b}, Pablo Cañizares^c, Manuel A. Rodrigo^c, Cristina Saez^c

*^a Electrochemistry and Nanotechnology Laboratory, Research and Technology Institute
- ITP, Aracaju, SE, Brazil*

*^b Processes Engineering Post-graduation - PEP, Universidade Tiradentes, 49037-580
Aracaju, SE, Brazil*

*^c Chemical Engineering Department, Universidad de Castilla-La Mancha, Campus
Universitario, Ciudad Real, Spain*

* Author to whom all correspondence should be addressed (cristina.saez@uclm.es)

Abstract

This work is focused on testing a novel MMO-Ti/RuO₂IrO₂ electrode to reduce the dangerousness of hospital urines polluted with antibiotics. To do this, this electrode was used as anode in electro-Fenton (EF) and photoelectro-Fenton (PhEF) processes, and results were compared with those obtained using boron doped diamond (BDD) as anode, as well as those obtained by a conventional Fenton oxidation. The performance of treatment processes was evaluated in terms of antibiotic removal, toxicity (using standardized method with *Vibrio fischeri*) and antibiotic resistance of treated urine (using *Enterococcus Faecalis* as target bacterium). Results demonstrate that Penicillin G degrades in the following sequence: Fenton < EF < PhEF. The best results were found for the MMO-PhEF, capable of removing 100% of the antibiotic, reducing toxicity by 96%, and reaching complete removal of the antibiotic activity. Tests were also carried out with BDD for the sake of comparison and results point out the higher convenience of the new electrode in terms of a more adequate use of energy because the effluents generated can be further degraded in urban wastewater treatment plant. Because of that, MMO-RuO₂-IrO₂ emerges as a promising cost-effective material for the pre-treatment of hospital urine.

Keywords

Urine; Metal mixed oxides; Penicillin G; Toxicity; Antimicrobial activity

1. Introduction

Advanced oxidation processes (AOPs) have shown high potential for the removal of organic compounds due to the *in situ* generation of highly reactive and nonselective hydroxyl radical ($\bullet\text{OH}$, $E^\circ = 2.8 \text{ V/SHE}$) capable to led complete mineralization of many types of pollutants [1–3]. Among AOPs, electrochemical advanced oxidation processes (EAOPs) have attracted considerable attention due to their important advantages, as compared with traditional AOPs, because they: i) can manage a wide range of pollutants and concentrations; ii) can generate H_2O_2 *in situ*; iii) operate at mild conditions and can be easily scalable, and iv) are considered environmentally friendly [4].

In these electrochemical based processes, the oxidation capacity to remove organic pollutants depends, among other factors, on the anode material used [5]. For example, Boron doped diamond (BDD) anodes, widely studied in the literature[6], are able of obtaining complete mineralization of many types of wastewater due to their greater O_2 potential, that allows the electrogeneration of a high amount of hydroxyl radical. The excellent results obtained[7] have postulated this electrode as one of the most suitable for the treatment of water contaminated with refractory and difficultly oxidizable compounds. However, in some cases the treatment target is not the complete oxidation of organic load but the selective degradation of pollutants, or their conversion into compounds with greater biodegradability that can be later eliminated by conventional lower-cost treatment techniques. In this point, metal mixed oxide(MMO) anodes have attracted attention of researchers due to their excellent electrocatalytic properties: high production of oxygen and hydrogen by electrolysis in water [8], excellent mechanical stability, long service lifetime and electrocatalytic activity. These electrocatalytic properties are related to the type of interaction between the electrode and hydroxyl radicals in the oxidation of water and may explain the differences observed in selectivity

and efficiency during the oxidation process with both types of anode material: MMO is less efficient in oxidizing organic matter but more appropriate in converting organic contaminants into simpler molecules [9]. BDD is considered a non-active electrode, where the hydroxyl radicals are physically attached to the electrode surface (weak interaction), while the MMO is reported as an active anode, because the hydroxyl radicals are chemisorbed on the electrode surface (strong interaction) and, as it is well-known, the weaker is the $M(\bullet OH)$ interaction, the higher is the amount of hydroxyl radicals available and the corresponding efficiency for oxidizing organics [10]. Cotillas et al. [11] studied the removal of penicillin G in urine by comparing MMO *versus* DDB anodes and observed a complete mineralization of organic load when using DDB and a selective oxidation of antibiotic (and not of organic compounds present in urine) when using MMO.

This is very relevant in the search of strategies to increase the biodegradability and to reduce the hazardousness of aqueous wastes, and it has encouraged the search of novel MMO formulation, as well as the combination of technologies to make their use in potential applications for wastewater deal with. At this point, many studies have focused on the use of electro-oxidation in combination with Fenton process [1], resulting in the well-known electro-fenton process (EF). Fenton's oxidation is one of the best-known metal catalyzed oxidation reactions of water-miscible organic compounds. The mixture of $FeSO_4$ and H_2O_2 at low pHs, results in Fe^{2+} catalytic decomposition of H_2O_2 [12]. So acting, in production hydroxyl radicals which have extremely high oxidizing ability and can oxidize hard to decompose organic compounds in a short time [7]. In EF, the amount of Fe^{+2} added to the system is lower as compared to traditional Fenton, due to the regeneration of Fe^{+2} in the cathode [13]. Additionally, the in-situ production of hydrogen peroxide by the reduction of oxygen at the cathode [14] has demonstrated to be critical to

improve the efficiency of degradation process. In this point, for a competitive implementation of electrofenton process, the selection of appropriate cathodic materials is crucial [15]. The use of carbonaceous cathodes has been largely studied: graphite or graphite felt [16,17], reticulated vitreous carbon [18,19], carbon-polytetrafluoroethylene (PTFE) electrode [7,20] and gas diffusion electrode (GDE) [21]. According to Pérez *et al.* [22], the mixture between carbon black and polytetrafluoroethylene (CB/PTFE) applied to the carbon felt is one of the key point to promote a quick and efficient production of hydrogen peroxide. Once formed, H_2O_2 must be activated and its oxidation in the anode must be minimized

In the search of synergies, the combination of UV irradiation with EF (PhEF process) and the promotion of some photo-assisted reactions have been also evaluated including the: i) photolysis of H_2O_2 molecules producing hydroxyl radicals, ii) photodegradation of Fe (III) complexes with organic compounds and iii) photodegradation of photoactive $Fe(OH)^{2+}$ [23]. In addition, UVC light can contribute to the direct photolysis of aromatic molecules. Santos and co-workers[24] studied the degradation of clopyralid using EF and PhEF using different anodes. In both processes, the mineralization of the compound was obtained; however, the efficiency of the process and the synergy of the technologies was higher when using PhEF. Similarly, Zhang *et al.*[25] studied the degradation of the antibiotic cephalexin through EF and PhEF processes using different cathodes. They studied different conditions and, in all cases,, the PhEF processes showed a faster breakdown of the pollutant than the EF. Such behavior was attributed to the photodecomposition of photoactive species.

With this background, this work aims to study the feasibility of a promising microwave-prepared MMO-Ti/RuO₂IrO₂ [26] in Fenton-based electrochemical processes and to compare the results with those obtained with a commercial BDD anode. Then, the

Fenton, electro-Fenton and photoelectron-Fenton processes are evaluated for the particular case of reducing the dangerousness of hospital urines which is related to the presence of pharmaceuticals [11,27]. Therefore, this work tries to shed light on the search of novel electrode material for the conversion of potentially hazardous pharmaceuticals into other less hazardous intermediate compounds that can be discharged to the sewage network without generating problems in urban wastewater treatment plants [28,29] and without contributing to the proliferation of antibiotic-resistant bacteria. In this work, Penicillin G (PenG) was selected as model of pharmaceutical due to it is one of the most frequently used antibiotics (beta-lactam antibiotic) and has an antibacterial activity associated with the interruption of the synthesis of bacterial cell walls [30,31]. The performance of treatment processes was evaluated in terms of antibiotic removal, toxicity (using standardized method with *Vibrio fischeri*) and antibiotic resistance of treated urine (using *Enterococcus Faecalis*). Thus, the results obtained will help to understand the behavior of the MMO-Ti/RuO₂IrO₂ anode in a complex matrix in which inorganic and organic compounds compete for oxidation and will also help to clarify the behavior of the electrodes to transform recalcitrant pollutants into less dangerous compounds in a selective way.

2. Experimental

2.1 Chemicals

Penicillin G, urea, creatinine and uric acid purchased from Sigma Aldrich® were of analytical grade (99%). Heptahydrate ferrous sulfate used as catalyst was also purchased from Panreac®. The other chemicals used were of analytical grade, purchased from Sigma-Aldrich® and were used as received. Methanol and formic acid used for the mobile phase of High- Performance Liquid Chromatography (HPLC) were purchased from

Sigma-Aldrich®. All aqueous solutions were prepared using high-purity water (Millipore Milli-Q system, resistivity > 18 MΩ). The synthetic urine used consists of a mixture of organic species and inorganic salts, a composition previously used by Dbira et al. [32].

2.2 Experimental set-up

All the experiments were performed at a laboratory scale with a cylindrical, open, and undivided three-electrode cell of 0.15 L capacity with a jacket recirculation of water thermostated at 25 °C. . BDD film, 200 ppm boron doping, supplied by Adamant Technologies or mixed metal oxide of Ti/Ru_{0.5}Ir_{0.5}O₂ prepared by microwave heating were employed. Their size was 4 cm². As cathode, a 60 cm² the modified carbon felt prepared according to a procedure described by Pérez et al. [22], was placed on the inner wall of the cell according to experimental set-up proposed for Özcan et al. [33] and fed with air bubbled 10 min prior each experiment. All the experiments were carried out at the constant current of 120 mA cm⁻² provided by an Autolab (Metrohm - Pensalab) PGSTAT302N potentiostat/galvanostat, controlled by software Nova 2.1. The solutions were stirred with a magnetic bar at 500 rpm for homogenization.

The PhEF were conducted by irradiating the solution with a 9W UVC lamp ($\lambda_{\text{max}} = 254 \text{ nm}$) that was immersed in the solution. The Fenton, EF, and PhEF process at 0.12 A degraded solutions containing 50 mg L⁻¹ of Penicilin G in urine. All the experiment, 0.5 mM Fe²⁺ was added to the solution as a catalyst of the Fenton's reaction.

2.3 Analytical techniques

The concentration of the Penicillin G was quantified by HPLC (Agilent 1260 series), equipped with an Eclipse Plus C-18 column (4.6mm x 100mm; 3.5μm). The mobile phase was a mixture of 50% methanol and 50% acidified water (formic acid 0.1%) at a flow

rate of 0.6 mL min^{-1} with the detection achieved at 220 nm. The injection volume was $20.0 \text{ }\mu\text{L}$, the column temperature was set to $30 \text{ }^{\circ}\text{C}$, and the retention time was around 2.4 min. Additionally, the mobile phase for the determination of uric acid consisted of 2% acetonitrile/98% aqueous solution with 0.1% of formic acid. A flow rate of 1 mL min^{-1} and a DAD detection of 292 nm were used. The injection volume was $10 \text{ }\mu\text{L}$ (retention time: 2.7 min).

Urea concentration was determined by a spectrophotometric method using UV-1700 Spectrophotometer (Shimadzu). This method is based on the yellow-green color produced when p-dimethylaminobenzaldehyde is added to urea in dilute hydrochloric acid solution[34]. Creatinine was determined by ion chromatography (IC) with a Metrohm 930 2 Compact IC Flex coupled to a conductivity detector. Anions were also analyzed by using the column Metrosep A Supp 7 with a mobile phase of 85:15 v/v 3.6 mmol dm^{-3} Na_2CO_3 /acetone and flow rate of 0.8 mL min^{-1} . Cations were analyzed by using the column Metrosep C6 250 with a mobile phase of 1.7 mmol dm^{-3} HNO_3 and 1.7 mmol dm^{-3} 2,6-pyridinedicarboxylic acid and flow rate of $0.9 \text{ cm}^3 \text{ min}^{-1}$. The total organic carbon (TOC) concentration was monitored using a TOC analyzer Multi N/C 3100 Analytik Jena. Chemical oxygen demand (COD) analyses were conducted by using a Spectroquant[®] test CSB from Merck[®].

2.4 Microbiological techniques

The toxicity assessment was carried out through the bioluminescence of the marine bacteria *Vibrio Fisheri*. The measurements were made using the Microtox[®], following the guidance of the protocol provided by the manufacturer. Before the tests, the samples were osmotically adjusted. Thus, the emitted bioluminescence of *vibrio fisheri*

was recorded after two different times of exposure to the collected aliquots, which were 5 and 15 minutes, and subsequently compared with the reference sample.

The antibiotic resistance of Penicillin G was measured by the number of colonies forming units (CFU) of the *Enterococcus Faecalis* bacteria using μ -Trac®4200. Initially, the microorganisms were spread on agar plates and incubated at 37°C for 24 h. Subsequently, the urine matrix was contaminated with 10^6 - 10^7 CFU of *E. faecalis*, and a blank sample was measured to control the initial concentration of microorganisms. Finally, the treated samples were added to the blank matrix (containing *E. faecalis*), in different concentrations (without dilution, 1:10, 1: 100 and 1: 1000), and kept under stirring for 3 h at 37 ° C. Finally, aliquots were transferred to a culture medium and taken to the equipment to perform the CFU count.

It is important to emphasize that the hazardousness of urine is related both to the toxicity of drugs and their metabolites and to the resistance that certain antibiotics can produce when bacteria mutate in response to their use and accumulation in the environment. To monitor the risk of the urine after the treatment two different microbiological tests were employed: analysis of toxicity (based on the use of the bioluminescent marine bacterium *Vibrio Fisheri*) and analysis of the antibiotic resistance (based on the use of *Enterococcus Faecalis* as target bacteria). These methods inform about the response of two different bacteria to compounds contained in the urine matrix and, therefore, they give complementary information. That is, toxicity tests quantify the decrease in the bioluminescence produced by the inhibition of enzymatic activity of *Vibrio fischeri* that is related to the toxic organics and/or oxidants present in the solution, but it is not specific to any target compound. On the other hand, *Enterococci* are intrinsically resistant to many agents, but they exhibit a great sensitivity to PenG. Therefore, a decrease in the *Enterococcus* population informs about the remaining

antibiotic effect of the treated urine that is related to the presence of penicillin G and maybe to its possible reaction intermediates. All this information, together with the physical-chemical characterization, will allow to establish the ideal operational conditions to reduce the chemical risk of hospital urine and the possible mutation of bacteria as a response to the environment.

3. Results and discussion

To evaluate the use of MMO-Ti/RuO₂IrO₂ for reducing the dangerousness of urine polluted with antibiotics, ElectroFenton (EF) and PhotoelectroFenton (PhEF) tests were carried with synthetic urine containing 50 mg L⁻¹ of Penicillin G. Figure 1 shows the inhibition percentage (estimated by the toxicity test) of the urine treated by EF and PhEF processes at 30 mA cm⁻² and using MMO-RuO₂IrO₂ as anode. Results are compared with those obtained using boron doped diamond (BDD) as anode, and with those attained by conventional Fenton oxidation. In all cases, plotted data correspond with urine treated for 2 hours.

Figure 1. Percentage of inhibition of *Vibrio fischeri* of samples of Penicillin G (50 mg L⁻³) in synthetic urine treated by Fenton, EF and PhEF using BDD or MMO anodes.

As can be observed, the initial synthetic urine polluted with 50 mg L⁻¹ of Penicillin G inhibits around 95 % of the activity of *Vibrio fischeri*, indicating that one or more species contained in the urine are toxic to the target bacteria. The test carried out to synthetic urine without antibiotic did not inhibit the activity of *Vibrio fischeri* (less than 2% of inhibition), indicating that the 95 % of inhibition observed in the polluted urine is related to PenG. After 2h of treatment, inhibition percentage decreases more than 80 %, and, as can be observed, this decrease depends on the treatment process. Urine treated by Fenton process presents inhibition percentage around 17 %, while urine treated EF and PhEF present inhibition percentage lower than 10 %. In both electrochemical based processes, a clear effect of the anode material is observed and the use of BDD seems to lead to more toxic treated urine. This indicates that the degradation point reached is

different, and that the concentration or nature of potentially toxic species (organics or inorganics) after 2 hours of treatment with each technology is not the same. That is, the organic intermediates generated (and accumulated in the reaction system) from the degradation of PenG or urine components can be different in each case, as well as the oxidants formed from the oxidation of inorganic species contained in urine (chloride, sulfate, phosphate or carbonates) [12].

Using MMO anodes, chlorides can be electrochemically oxidized to form hypochlorite, but the formation of high concentration of highly oxidized chlor-species (chlorate or perchlorate) is not expected. On contrary, it is well-known [35–37] that the use of BDD favors the formation of highly oxidized chlorine species, and this could explain the worst results obtained in terms of toxicity [38]. Figure 2 shows the concentration of chloro-species during the treatment of synthetic urine polluted with PenG by the different Fenton-based technologies. Initially, chloride ions decrease rapidly to form hypochlorite and chloramines (by reaction between hypochlorite and ammonium present in urine matrix, *break point reaction*), but for higher electrical charge passed the accumulation of chlorate and perchlorate in the reaction system starts to be relevant, mainly in the case of using BDD as anode. Note that when using BDD, chlorate is in a much higher concentration than when MMO is used. Additionally, no perchlorate was detected when working with MMO. These results agree with those reported in literature [35] and they may be a result of the additional hydroxyl radicals provided by BDD. Bergmann et al. [36] reported that electrolysis of chlorine solutions with MMOs leads to the formation of chlorate, but no perchlorate was detected. Santos et al. [24], also demonstrated that when using MMO in the EF and PhEF processes, chlorates were formed, while no perchlorates were found, and that the irradiation of UV light favors the accumulation of both chloro-species in the reaction media. The formation of these species

can also have an important impact in the aim of decreasing the dangerous of hospital urines.

Figure 2. Evolution chloro-species formed during degradation of 50 mg L⁻¹ of PenG by different Fenton-based process.

However, to exclude as much as possible the contribution of oxidants and to minimize toxicity inhibition related to highly toxic inorganic substances, sodium sulfite was added to each urine sample (with a ratio of sodium sulfate mol / mol oxidants quantified by titration I/I₂) prior to toxicity analysis. Thus, the inhibition observed should be mainly related to the toxicity of organic that remain in the treated urine. Additionally, the antibiotic resistance of the same treated urines was carried.

Figure 3 shows the population of the target bacterium that remain in the solution after the antibiotic resistance tests. In all cases, the initial population of pure culture of *E. Faecalis* was 10⁷ UFC mL⁻¹ and each sample was measured in quadruplicate: (without dilution and dilution 1:10, 1: 100 and 1: 1000). If the treated urine still presents antibiotic

effect, the population of *Enterococcus* should decrease, indicating its potential risk of ecotoxicity.

Figure 3. Antibiotic effect of urine treated by different Fenton-based processes.

Dashed line: initial UFC.

In the test carried out with the synthetic urine polluted with 50 mg dm^{-3} the complete killing of the target bacterium was attained, confirming that the PenG is an appropriate antibiotic to treat infections related to *E. faecalis*. However, once urine is treated for 2 h, the antibiotic effect of the urine decreases and it is almost inexistent: the *E. faecalis* population remains around the initial log unit (10^7 UFC ml^{-1}). Only in the urine treated by Fenton oxidation, the population of *Enterococcus* decreases 2 log unit (till 10^5 UFC mL^{-1}). In the other cases, the antibiotic effect is almost nil and no relevant differences are observed, although, as in the case of toxicity, BDD seems to behave worse than MMO, and the potential risk of urine treated using diamond base electrode is slightly higher. The

observed differences may be related to the remaining concentration of antimicrobial agents: Pen G or its degradation products. Nevertheless, these results reveal the low risk of toxicity of these treated urines and, therefore, their release into the environment should not have any relevant adverse effect. When analyzing the microbiological data, it is possible to state that the use of MMO-RuO₂IrO₂ is viable, due to its excellent performance in the treatment of hospital wastewater.

To explain this, the concentration of PenG was also monitored. Figure 4 shows the degradation of Penicillin G by ElectroFenton (EF) and PhotoelectroFenton (PhEF) processes in urine media using both MMO-Ti/RuO₂IrO₂ and BDD. Results obtained by Fenton oxidation are also plotted. As can be observed, all Fenton-based processes exhibit excellent performance, reaching between 70-88 % of removal after 2 hours of treatment (1.6 Ah dm⁻³), and more than 98% drug removal after 8 h (6.4 Ah dm⁻³). However, the removal rate depends on the process: PhEF > EF > Fenton oxidation.

Figure 4. a) Penicillin G decay as a function of the treatment time by different Fenton-based process in urine matrix. b) Penicillin G decay kinetics as a function of treatment time by different Fenton-based processes in the urine matrix

The obtained results were adjusted to kinetic model and as can be observed in Figure 4b the degradation of Penicillin G fitted well to pseudo-first-order reaction kinetic. The observed kinetic constants are also summarized in Table 1. It is possible to observe not only one, but two regions of first-order kinetics for EF and PhEF, which suggests a change in the oxidation mechanism of organic matter when most of Pen G is degraded and converted into simpler organic intermediates, which seem to have different oxidation behavior. Comparing results, the kinetic constant of Penicillin G is lower in the EF than in PhEF, this indicates that the irradiation of UV light during electrochemical oxidation seems to promote the selectivity towards PenG degradation. The better performance of PhEF process can also be explained in terms of the enhancement of the oxidation ability by the photolysis of Fe(III)-carboxylate species [39]. The effect of anode used is less

Comentado [CSJ1]: Dejar las dos? O solo una?

Comentado [MARR2R1]: Poner la segunda como onset de la primera solo com eje de tempo y quitar la leyenda

relevant, although MMO anodes behave better than BDD one more time, and its use leads to a faster drug removal than diamond electrode.

Table 1. Penicillin G and mineralization percentages and observed kinetic constants for Penicillin G decay by different Fenton- based process in urine matrix.

Process	TOC _{removal} %	PenG _{removal} / %	k _{penG} / min ⁻¹	R ²	k _{penG} / min ⁻¹	R ²
Fenton	25.0	98.0	0.50	0.99	-	-
EF-MMO	54.0	99.0	0.80	0.99	0.35	1.00
EF-BDD	51.0	98.4	0.65	0.99	0.40	0.98
PhEF- MMO	68.0	100.0	1.34	0.99	0.75	0.95
PhEF - BDD	65.0	99.6	0.98	0.99	0.57	0.96

Additionally, to confirm how the degradation of organic compounds typically present in urine competes with Pen G oxidation [40,41] and how their degradations disturb the main aim (the decrease of the dangerousness of the antibiotic-polluted urine), the removal of urea, creatinine and uric acid during the Fenton-based processes were monitored as well as the removal of total organic carbon (TOC) and of chemical oxygen demand (COD). Figure 5 shows the removal of these organic compounds after 2 hours of treatment (part a) and of TOC and COD after 2 and 8 hours of treatment (part b) by Fenton, EF and PhEF processes of urine polluted with penicillin G. The obtained conclusions are like those gained from penicillin G removal: PhEF is more efficient than EF and Fenton oxidation. Uric acid is more easily oxidized than urea and creatinine, but the removal percentage attained is lower than that observed for Pen G (ranging from 70 to 88 %, as seen in Figure 5a). Then, although Pen G is in lower concentration than uric acid, creatinine and urea,

its degradation is favored and this opens the possibility of developing selective degradation technologies, with a great impact on the degradation of the hazardousness of hospital wastewater.

Figure 5. (a) Uric acid, creatinine, urea and TOC removal after 2h treatment using Fenton, EF and PhEF in synthetic urine solutions. (b) TOC and COD removal after 8h of treatment using Fenton, EF and PhEF in synthetic urine solution. Current density in electrochemical processes: 30 mA cm^{-2} . UV-light power in irradiated processes: 9 W . $\text{TOC}_0 = 800 \text{ mg L}^{-1}$ and $\text{COD}_0 = 398 \text{ mg L}^{-1}$

It overall parameters such as TOC and COD are analyzed (Figure 5b), it can be confirmed that Fenton oxidation is not able to attain the complete oxidation of these compounds to carbon dioxide, while electrochemical based technologies reach around 25 % of mineralization during the first 2 hours of treatment and this percentage increases up to 50 or 70 % in EF and PhEF, respectively, after 8 hours of treatment. In terms of COD, the removal percentages vary between 60 and 75 % in the electrochemical techniques tested. This confirms the conversion of raw organic in simpler molecules (most likely carboxylic acids).

Then, on base of all these results, it is possible to state that EF and PhEF with MMO electrodes are able to degrade selectively PenG and that after 2 hours of treatment

Comentado [CSJ3]: Estas dos figuras se podrían integrar en una de puntos con tres series: TOC a las 2 h, TOC a las 8h y COD a las 8 h. La eliminación de COD a las 2 h no está medida. Otra opción es no hablar de eliminación a las 8 h, y hablar solo a las 2h que es cuando están medida las técnicas bio y centrar ahí la discusión

Comentado [MARR4R3]: Dejar solo la segunda: resultados de tratamiento a las 8 horas.

Poner en a TOC a las 2 horas como si fuese un componente

the antimicrobial activity of the urine has almost disappeared and the treated urine could be safely merged with urban-type hospital wastewater. Figure 6 shows the energy consumption per unit of PenG removed required to attain this target point. As seen, this parameter appears to depend on the process applied and the anode material used. The values found showed that the energy consumption decreased when MMO was used as an anode. Such behavior occurs, probably due to the better conductivity of the catalytic coating. In addition, the slightly lower values of energy consumption found for PhEF (with 9 W UVC light) as compared to EF, regardless of anode material, may be an attractive option for treating hospital wastewater. The results obtained showed that, regardless of the technology used, the new MMO-RuO₂-IrO₂ anode can efficiently remove Pen G and completely eliminate the hazardousness of urine (toxicity and antibiotic activity) without losing energy in the degradation of biodegradable organics that can be further degraded in urban wastewater treatment plant. So, it appears as a promising cost-effective material for the pre-treatment of hospital urine.

Comentado [CSJ5]: Tal vez lo podíamos referir a reducción de inhibición o de actividad antimicrobiana, en vez de PenG ¿?

Figure 6. Specific energy consumption for the electroFenton (EF) and photo-electroFenton (PhEF) processes carried out at 30 mA cm^{-2} in urine by using MMO- RuO_2IrO_2 and BDD anodes.

Conclusions

Analyzing the results obtained in this research, the following conclusions are drawn:

- EF and PhEF with MMO- RuO_2IrO_2 and BDD anodes can be used to remove selectivity PenG from hospital urine.
- No toxic byproducts such as are generated in case of using MMO- RuO_2IrO_2 as anode.
- The removal rate depends on the process: $\text{PhEF} > \text{EF} > \text{Fenton oxidation}$. In terms of energy consumption, PhEF with MMO is the best technology to decrease the ecotoxicity risk of hospital urine polluted with Pen G.

4. References

- [1] M.A. Oturan, J.J. Aaron, Advanced oxidation processes in water/wastewater treatment: Principles and applications. A review, *Crit. Rev. Environ. Sci. Technol.* (2014). doi:10.1080/10643389.2013.829765.
- [2] M.S. Saghafeinia, S.M. Emadian, M. Vossoughi, Performances evaluation of Photo-Fenton process and Sonolysis for the treatment of Penicillin G formulation effluent, *Procedia Environ. Sci.* 8 (2011) 202–208. doi:10.1016/j.proenv.2011.10.033.
- [3] X. Zhou, D. Liu, Y. Zhang, J. Chen, H. Chu, Y. Qian, Degradation mechanism and kinetic modeling for UV/peroxydisulfate treatment of penicillin antibiotics, *Chem. Eng. J.* 341 (2018) 93–101. doi:10.1016/j.cej.2018.01.137.
- [4] R. Dewil, D. Mantzavinos, I. Poulios, M.A. Rodrigo, New perspectives for Advanced Oxidation Processes, *J. Environ. Manage.* 195 (2017) 93–99. doi:10.1016/j.jenvman.2017.04.010.
- [5] F. Sopaj, M.A. Rodrigo, N. Oturan, F.I. Podvorica, J. Pinson, M.A. Oturan, Influence of the anode materials on the electrochemical oxidation efficiency. Application to oxidative degradation of the pharmaceutical amoxicillin, *Chem. Eng. J.* 262 (2015) 286–294. doi:10.1016/j.cej.2014.09.100.
- [6] S.T. McBeath, D.P. Wilkinson, N.J.D. Graham, Application of boron-doped diamond electrodes for the anodic oxidation of pesticide micropollutants in a water treatment process: A critical review, *Environ. Sci. Water Res. Technol.* (2019). doi:10.1039/c9ew00589g.

- [7] J.F. Pérez, J. Llanos, C. Sáez, C. López, P. Cañizares, M.A. Rodrigo, Treatment of real effluents from the pharmaceutical industry: A comparison between Fenton oxidation and conductive-diamond electro-oxidation, *J. Environ. Manage.* 195 (2017) 216–223. doi:10.1016/j.jenvman.2016.08.009.
- [8] M. Wang, Z. Wang, X. Gong, Z. Guo, The intensification technologies to water electrolysis for hydrogen production – A review, *Renew. Sustain. Energy Rev.* 29 (2014) 573–588. doi:10.1016/j.rser.2013.08.090.
- [9] M. Shestakova, P. Bonete, R. Gómez, M. Sillanpää, W.Z. Tang, Novel Ti/Ta₂O₅-SnO₂ electrodes for water electrolysis and electrocatalytic oxidation of organics, *Electrochim. Acta.* 120 (2014) 302–307. doi:10.1016/j.electacta.2013.12.113.
- [10] M.A.Q. Alfaro, S. Ferro, C.A. Martínez-Huitle, Y.M. Vong, Boron doped diamond electrode for the wastewater treatment, *J. Braz. Chem. Soc.* (2006). doi:10.1590/S0103-50532006000200003.
- [11] S. Cotillas, E. Lacasa, M. Herraiz, C. Sáez, P. Cañizares, M.A. Rodrigo, The Role of the Anode Material in Selective Penicillin G Oxidation in Urine, *ChemElectroChem.* 6 (2019) 1376–1384. doi:10.1002/celec.201801747.
- [12] I. Sirés, E. Brillas, M.A. Oturan, M.A. Rodrigo, M. Panizza, Electrochemical advanced oxidation processes: Today and tomorrow. A review, *Environ. Sci. Pollut. Res.* 21 (2014) 8336–8367. doi:10.1007/s11356-014-2783-1.
- [13] S. Garcia-Segura, J. Anotai, S. Singhadech, M.C. Lu, Enhancement of biodegradability of o-toluidine effluents by electro-assisted photo-Fenton treatment, *Process Saf. Environ. Prot.* 106 (2017) 60–67. doi:10.1016/j.psep.2016.12.011.

- [14] G. Ren, M. Zhou, M. Liu, L. Ma, H. Yang, A novel vertical-flow electro-Fenton reactor for organic wastewater treatment, *Chem. Eng. J.* 298 (2016) 55–67. doi:10.1016/j.cej.2016.04.011.
- [15] J. Mejjide, J. Gómez, M. Pazos, M.A. Sanromán, Degradation of thiamethoxam by the synergetic effect between anodic oxidation and Fenton reactions, *J. Hazard. Mater.* 319 (2016) 43–50. doi:10.1016/j.jhazmat.2016.02.064.
- [16] E.E.S. Souza, A.F.T.O. Santos, I.M.D. Gonzaga, N.R. Alves, L.P. Costa, L.S.R. Silva, K.I.B. Eguluz, G.R. Salazar-Banda, E.B. Cavalcanti, Ruthenium-tin oxides-coated graphite felt: Enhanced active area and improved efficiency for the electrochemical generation of hydrogen peroxide, *Ceram. Int.* 41 (2015) 10293–10297. doi:10.1016/j.ceramint.2015.04.042.
- [17] F. Yu, M. Zhou, X. Yu, Cost-effective electro-Fenton using modified graphite felt that dramatically enhanced on H₂O₂ electro-generation without external aeration, *Electrochim. Acta.* 163 (2015) 182–189. doi:10.1016/j.electacta.2015.02.166.
- [18] H.C. Arredondo Valdez, G. García Jiménez, S. Gutiérrez Granados, C. Ponce de León, Degradation of paracetamol by advance oxidation processes using modified reticulated vitreous carbon electrodes with TiO₂ and CuO/TiO₂/Al₂O₃, *Chemosphere.* 89 (2012) 1195–1201. doi:10.1016/j.chemosphere.2012.07.020.
- [19] Y. Jin, Y. Shi, Z. Chen, R. Chen, X. Chen, X. Zheng, Y. Liu, Combination of sunlight with hydrogen peroxide generated at a modified reticulated vitreous carbon for drinking water disinfection, *J. Clean. Prod.* 252 (2020) 119794. doi:10.1016/j.jclepro.2019.119794.
- [20] J.F. Pérez, A. Galia, M.A. Rodrigo, J. Llanos, S. Sabatino, C. Sáez, B. Schiavo,

- O. Scialdone, Effect of pressure on the electrochemical generation of hydrogen peroxide in undivided cells on carbon felt electrodes, *Electrochim. Acta.* 248 (2017) 169–177. doi:10.1016/j.electacta.2017.07.116.
- [21] X. Yu, M. Zhou, G. Ren, L. Ma, A novel dual gas diffusion electrodes system for efficient hydrogen peroxide generation used in electro-Fenton, *Chem. Eng. J.* 263 (2015) 92–100. doi:10.1016/j.cej.2014.11.053.
- [22] J.F. Pérez, S. Sabatino, A. Galia, M.A. Rodrigo, J. Llanos, C. Sáez, O. Scialdone, Effect of air pressure on the electro-Fenton process at carbon felt electrodes, *Electrochim. Acta.* 273 (2018) 447–453. doi:10.1016/j.electacta.2018.04.031.
- [23] B.R. Garza-Campos, J.L. Guzmán-Mar, L.H. Reyes, E. Brillas, A. Hernández-Ramírez, E.J. Ruiz-Ruiz, Coupling of solar photoelectro-Fenton with a BDD anode and solar heterogeneous photocatalysis for the mineralization of the herbicide atrazine, *Chemosphere.* 97 (2014) 26–33. doi:10.1016/j.chemosphere.2013.10.044.
- [24] G. de O.S. Santos, K.I.B. Eguiluz, G.R. Salazar-Banda, C. Saez, M.A. Rodrigo, Testing the role of electrode materials on the electro-Fenton and photoelectro-Fenton degradation of clopyralid, *J. Electroanal. Chem.* (2020). doi:10.1016/j.jelechem.2020.114291.
- [25] Y. Zhang, A. Wang, S. Ren, Z. Wen, X. Tian, D. Li, J. Li, Effect of surface properties of activated carbon fiber cathode on mineralization of antibiotic cefalexin by electro-Fenton and photoelectro-Fenton treatments: Mineralization, kinetics and oxidation products, *Chemosphere.* 221 (2019) 423–432. doi:10.1016/j.chemosphere.2019.01.016.
- [26] I.M.D. Gonzaga, A.R. Dória, V.M. Vasconcelos, F.M. Souza, M.C. dos Santos,

- P. Hammer, M.A. Rodrigo, K.I.B. Eguiluz, G.R. Salazar-Banda, Microwave synthesis of Ti/(RuO₂)_{0.5}(IrO₂)_{0.5} anodes: Improved electrochemical properties and stability, *J. Electroanal. Chem.* 874 (2020) 114460. doi:10.1016/j.jelechem.2020.114460.
- [27] M. Fernández, M. Fernández, A. Laca, A. Laca, M. Díaz, Seasonal occurrence and removal of pharmaceutical products in municipal wastewaters, *J. Environ. Chem. Eng.* 2 (2014) 495–502. doi:10.1016/j.jece.2014.01.023.
- [28] P. Verlicchi, A. Galletti, M. Petrovic, D. Barceló, M. Al Aukidy, E. Zambello, Removal of selected pharmaceuticals from domestic wastewater in an activated sludge system followed by a horizontal subsurface flow bed - Analysis of their respective contributions, *Sci. Total Environ.* 454–455 (2013) 411–425. doi:10.1016/j.scitotenv.2013.03.044.
- [29] J. Talvitie, A. Mikola, A. Koistinen, O. Setälä, Solutions to microplastic pollution – Removal of microplastics from wastewater effluent with advanced wastewater treatment technologies, *Water Res.* 123 (2017) 401–407. doi:10.1016/j.watres.2017.07.005.
- [30] J. Heesemann, [Mechanisms of resistance to beta-lactam antibiotics], *Infection*. (1993).
- [31] Michael I. Page, The Mechanisms of Reactions of β -Lactam Antibiotics, *Acc. Chem. Res.* 17 (1984) 144–151.
- [32] S. Dbira, N. Bensalah, A. Bedoui, P. Cañizares, M.A. Rodrigo, Treatment of synthetic urine by electrochemical oxidation using conductive-diamond anodes, *Environ. Sci. Pollut. Res.* 22 (2015) 6176–6184. doi:10.1007/s11356-014-3831-6.

- [33] A. Özcan, N. Oturan, Y. Şahin, M.A. Oturan, Electro-fenton treatment of aqueous clopyralid solutions, *Int. J. Environ. Anal. Chem.* 90 (2010) 478–486. doi:10.1080/03067310903096011.
- [34] G.W. Watt, J.D. Chrisp, Spectrophotometric Method for Determination of Urea, *Anal. Chem.* (1954). doi:10.1021/ac60087a006.
- [35] M.E.H. Bergmann, J. Rollin, T. Iourtchouk, The occurrence of perchlorate during drinking water electrolysis using BDD anodes, *Electrochim. Acta.* (2009). doi:10.1016/j.electacta.2008.09.040.
- [36] M.E.H. Bergmann, J. Rollin, A.S. Koparal, Chlorate and perchlorate – new criterions for environmentally-friendly processes in Advanced Oxidation, *Water Pract. Technol.* (2010). doi:10.2166/wpt.2010.031.
- [37] M.E.H. Bergmann, A.S. Koparal, T. Iourtchouk, Electrochemical Advanced oxidation processes, formation of halogenate and perhalogenate species: A critical review, *Crit. Rev. Environ. Sci. Technol.* (2014). doi:10.1080/10643389.2012.718948.
- [38] G.O.S. Santos, K.I.B. Eguiluz, G.R. Salazar-Banda, C. Sáez, M.A. Rodrigo, Understanding the electrolytic generation of sulfate and chlorine oxidative species with different boron-doped diamond anodes, *J. Electroanal. Chem.* 857 (2020) 113756. doi:10.1016/j.jelechem.2019.113756.
- [39] S. Garcia-Segura, Á.S. Lima, E.B. Cavalcanti, E. Brillas, Anodic oxidation, electro-Fenton and photoelectro-Fenton degradations of pyridinium- and imidazolium-based ionic liquids in waters using a BDD/air-diffusion cell, *Electrochim. Acta.* (2016). doi:10.1016/j.electacta.2016.03.057.

- [40] S. Cotillas, E. Lacasa, C. Sáez, P. Cañizares, M.A. Rodrigo, Electrolytic and electro-irradiated technologies for the removal of chloramphenicol in synthetic urine with diamond anodes, *Water Res.* 128 (2018) 383–392. doi:10.1016/j.watres.2017.10.072.
- [41] S.D. Jojoa-Sierra, J. Silva-Agreto, E. Herrera-Calderon, R.A. Torres-Palma, Elimination of the antibiotic norfloxacin in municipal wastewater, urine and seawater by electrochemical oxidation on IrO₂ anodes, *Sci. Total Environ.* 575 (2017) 1228–1238. doi:10.1016/j.scitotenv.2016.09.201.
- [42] P. Villegas-Guzman, F. Hofer, J. Silva-Agreto, R.A. Torres-Palma, Role of sulfate, chloride, and nitrate anions on the degradation of fluoroquinolone antibiotics by photoelectro-Fenton, *Environ. Sci. Pollut. Res.* 24 (2017) 28175–28189. doi:10.1007/s11356-017-0404-5.