Chemico-Biological Interactions 291 (2018) 162–170

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

Electrochemical remediation of amoxicillin: detoxification and reduction of antimicrobial activity

Lara Barroso Brito[a,1], Luane Ferreira Garcia[a,1], Marcos Pereira Caetano[a], Germán Sanz Lobón[b], Mayk Teles de Oliveira[a], Rhaul de Oliveira[c], Ieda Maria Sapateiro Torres[a], Alfonso Yepez[d], Boniek Gontijo Vaz[b], Rafael Luque[d], Cesar Koppe Grisolia[e], Marize Campos Valadares[a], Eric de Souza Gil[a], Gisele Augusto Rodrigues de Oliveira[a,f,*]

[a] Faculty of Pharmacy, Federal University of Goiás (UFG), Goiânia, Goiás, Brazil
[b] Chemistry Institute, Federal University of Goiás, Goiânia, Goiás, Brazil
[c] Faculty of Pharmaceutical Sciences, University of São Paulo, USP, São Paulo, SP, Brazil
[d] Department of Organic Chemistry, University of Córdoba, Córdoba, Andaluzia, Spain
[e] Biological Sciences Institute, University of Brasília (UnB), Brasília, Distrito Federal, Brazil
[f] National Institute for Alternative Technologies of Detection, Toxicological Evaluation and Removal of Micropollutants and Radioactives (CNPq: INCT-DATREM), UNESP, Institute of Chemistry, Araraquara, SP, Brazil

ARTICLE INFO

Keywords:
Antibiotic
Zebrafish embryo
Extended exposure
Electrochemical oxidation
Bacterial resistance

ABSTRACT

Amoxicillin (AMX) is one of the most commonly prescribed antibiotics around the world to treat and prevent several diseases in both human and veterinary medicine. Incomplete removal of AMX during wastewater treatment contributes to its presence in water bodies and drinking water. AMX is an emerging contaminant since its impact on the environment and human health remains uncertain. This contribution was aimed to evaluate the electrochemical oxidation (EO) of AMX using different anodes in tap water, NaCl or Na_2SO_4 solutions and to evaluate the potential toxicity of remaining AMX and its by-products on zebrafish early-life stages. Chemical intermediates generated after EO were determined by mass spectrometry and their resulting antimicrobial activity was evaluated. AMX did not induce significant mortality in zebrafish during extended exposure but affected zebrafish development (increased body length) from 6.25 mg/L to 25 mg/L and inhibited enzymatic biomarkers. Carbon modified with titanium oxide (TiO_2@C) anode achieved complete AMX removal in just a few minutes and efficiency of the supported electrolytes occurred in the following order: 0.1 M NaCl > 0.1 M Na_2SO_4 > 0.01 M NaCl > tap water. The order of potential toxicity to zebrafish early life-stages related to lethal and sublethal effects was as follows: 0.1 M Na_2SO_4 > 0.1 M NaCl > 0.01 M NaCl = tap water. Additionally, the EO of AMX using TiO_2@C electrode with 0.01 M NaCl was able to inhibit the antimicrobial activity of AMX, reducing the possibility of developing bacterial resistance.

1. Introduction

Pharmaceutical drugs are a class of emerging contaminants that reach the environment through several anthropogenic sources [1]. The levels of such micropollutants found in hospital and domestic sewage systems range from $ng.L^{-1}$ to $\mu g.L^{-1}$, being generally higher for pharmaceutical companies. Owing to the inherent biological activity, the increasing disposal of pharmaceuticals may represent a risk for the environment and human health. The concern about such class of micropollutants is due to the disposal and dangerousness scale [2,3].

In this context, antibiotics have been detected in rivers, lakes, groundwater, drinking water, sea coastal water, urban effluents, and treatment plants [4–6]. They are widely used in human and veterinary medicines to treat and prevent bacterial infections, as well as in feed additives used in fish farms and livestock production as growth promoters or in modern agriculture to prevent bacterial crop damage [7–9].

Despite the low environmental levels due to their reasonable stability and continued release, antibiotics are classified as “pseudo persistent” contaminants [8,10]. Amoxicillin (AMX) is the most commonly antibiotic prescribed in Brazil [11]. It is known that more than 80% of AMX is excreted unchanged in urine and faeces of humans and animals

* Corresponding author. Rua 240, s/n, Setor Leste Universitário, 74605, Goiânia, Goiás, Brazil.
E-mail addresses: gisele23.rodrigues@hotmail.com, gaugusto@ufg.br (G.A. Rodrigues de Oliveira).
[1] These authors participated equally in this research.

https://doi.org/10.1016/j.cbi.2018.06.017
Received 7 February 2018; Received in revised form 18 May 2018; Accepted 13 June 2018
Available online 18 June 2018
0009-2797/

[12,13] and conventional waste water treatment plants are relatively inefficient to achieve their complete removal [6,8,9].

The scientific literature data reporting potential effects of AMX on aquatic life are scarce, probably because in most cases these have been associated with EC_{50} values > 1000 mg/L, thus indicating low acute toxicity [7,13,14]. In fact, the maximum concentration of AMX reported in aquatic systems is only of 6.94 μg/L, but the major concern relates to the long-term exposure associated with the possibility of generating chronic effects and resistance genes [5,6,15–18].

Considerable efforts have been made to develop novel remediation strategies for this pharmaceutical class in order to overcome the antibiotic-resistant bacteria problem and potential toxicity to non-target organisms. Electrochemical remediation of pollutants has several advantages including versatility to remove different contaminants, low cost, high energy efficiency, easy handling due to simple equipment and safety to operate in mild conditions [19]. Electrochemical oxidation (EO) is a promising technology already extensively applied to wastewater cleaning, in which electro-generated radicals lead to the complete mineralization of organic contaminants [19–24].

Despite high efficiency of some electrodes such as BDD and Ti/PbO_2, their cost and contamination risk by heavy metals due to chemical leaching from the anode, respectively, make them unfeasible in practical applications [23]. The use of carbon and titanium oxide electrodes could be an alternative of decreasing costs and increasing the environmental safety. Moreover, the efficiency of EO remediation is directly related to the electrode material, the electroactive surface area and the applied current density, whereas concerning to the medium, the pH has major influence accordingly to the contaminant type [25]. In turn, minimum system conductivity is required, thus suitable amounts of NaCl and Na_2SO_4 salts are commonly added into the medium [19,21–23].

Based on ongoing research efforts from the group, the aim of this work was to evaluate the effects of AMX on the development and biochemical activity of zebrafish (*Danio rerio*) early-life stages after 7 days of exposure and to perform EO remediation of AMX with different anodes, carbon (C), titanium (Ti) and carbon modified with titanium oxide (TiO_2@C) in tap water, NaCl or Na_2SO_4 solutions. Generated by-products in such media were monitored by mass spectrometry and potential toxicity was evaluated by zebrafish embryo tests. The antimicrobial activity of remaining AMX after EO remediation was monitored using a simple broth microdilution technique.

2. Material and methods

2.1. Test substances

Amoxicillin trihydrate (AMX; CAS: 61336-70-7; 98,7%) was purchased from Sigma–Aldrich (St Louis, MO, USA) and used in all assays as purchased. For Fish Embryo Extended Toxicity (FEET) Test and biochemical analyses, a stock solution was carefully prepared by dilution of AMX in deionized water, kept refrigerated and protected from light. Test solutions were prepared immediately before beginning the test by successive dilutions of the stock solution using maintenance water at 0.75, 1.5, 3.0, 6.25, 12, 25, 50 and 100 mg/L of AMX. To evaluate the EO efficiency, a stock solution of AMX at 12 mg/L was prepared with different electrolytes supported: 0.1 M of Na_2SO_4, 0.01 and 0.1 M of NaCl or tap water. To determine the antimicrobial activity before and after EO process, a stock solution of AMX at 1200 mg/L was prepared in deionized water.

2.2. Fish embryo extended toxicity (FEET) tests

Adult male and female zebrafish (*D. rerio*) were kindly provided by the zebrafish facility (ZebTec Tecniplast) at the Institute of Biology, University of Brasília and kept in separate tanks (ethical approval UFG Nº 102/2014). Fish were maintained in a Rack Hydrus (Alesco) recirculating system using water filtered by reverse osmosis, where water passes through several levels of filtration (activated carbon filters and biological filters) and is then disinfected by UV light and automatically adjusted for pH and conductivity. The temperature is maintained at 26.0 ± 1 °C, conductivity at 750 ± 50 μS, pH at 7.5 ± 0.5 and dissolved oxygen equal to or above 95% saturation. Nitrate, nitrite and ammonia are regularly monitored. As previously mentioned, this water was used to prepare the test solutions of all assays performed. Adult organisms were fed with commercial dry flake food (TetraMin Tropical Flakes®) and live brine shrimp. On the day of the test, zebrafish eggs were collected about 30 min after natural mating, rinsed in water and examined under a stereomicroscope (Bel Photonics STM PRO). Unfertilized or damaged eggs were discarded. The fertilization success was checked, and only eggs batches with a minimum fertilization rate of 90% were used. The fish embryo toxicity test with zebrafish (*D. rerio*) was carried out according to OECD guideline 236 [26] with extended exposure time for 7 days post-fertilization (dpf) to evaluate the subchronic effects of AMX on zebrafish early-life stages. Twenty fertilized eggs per concentration were randomly selected and carefully distributed to a 24-well plate, filled with 2 mL of different concentrations of AMX (0.75, 1.5, 3.0, 6.25, 12, 25, 50 and 100 mg/L) or negative (CN; maintenance water) and positive controls (PC: 3,4-dichloroaniline at 4.0 mg/L). The test was performed in triplicate in a climate chamber at 26 ± 1 °C and 12-h light. Neither food nor aeration was provided during the assays. Embryo development was assessed at 24, 48, 72, 96, 120, 144 and 168 h post-fertilization (hpf) using a stereomicroscope. The distinction between normal and abnormal embryonic development was established according to the zebrafish development descriptions reported by Kimmel et al. [27]. Lethal (egg coagulation, no somite formation, non-detachment of the tail from yolk sac and no heart beating) and sublethal (effects on the eye and body pigmentation, absorption of the yolk sac, hatching rate, swimming bladder inflation, otolith, presence of edemas and blood accumulation, tail deformities and body length) parameters were observed and reported.

2.2.1. Biochemical analyses

Catalase (CAT), glutathione-S-transferase (GST) and lactate dehydrogenase (LDH) activities were determined in zebrafish larvae exposed to 0.75, 1.5, 3.0, 6.25, 12, 25, 50 and 100 mg/L of AMX after 168 h of exposure. On the day of enzymatic analyses, a total of three samples per concentration (each sample composed of 7 larvae pool) were defrosted on ice, homogenized and centrifuged (4 °C, 10,000 g, 20 min) in order to isolate the post-mitochondrial supernatant (PMS) fraction posteriorly used as enzyme extract for activity determinations made by spectrophotometrically (Thermo Scientific Multiskan Spectrum, USA). Biomarker determinations were performed based on Oliveira et al. [7], in which CAT activity was measured at 240 nm by monitoring (for 3 min) the decrease of absorbance due to degradation of hydrogen peroxide (H_2O_2), as described by Clairborne [28]. GST activity was determined at 340 nm by monitoring the absorbance increase (for 5 min) following the methodology described by Habig and Jakoby [29] and Frasco and Guilhermino [30]. LDH activity was measured at 340 nm by monitoring (for 5 min) the absorbance decrease due to the oxidation of NADH, according to Vassault [31] and Diamantino et al. [32]. Enzymatic activities were determined in quadruplicate and expressed as nanomoles of hydrolyzed substrate per minute per mg of protein (mmol/min/mg of prot). Protein concentration in samples was determined in quadruplicate according to the Bradford method [33] at 595 nm, using γ-globulin as a standard.

2.3. Electrochemical oxidation (EO)

The anodes used for EO were Carbon cylinder (C; 3.5 × 0.5, length x diameter cm; geometric area = 5.89 cm^2), Titanium wire (Ti^0; 10.0 × 0.2, length x diameter cm; geometric area = 6.34 cm^2) and C modified (TiO_2@C; 3.0 × 0.5, length x diameter cm; geometric

area = 5.10 cm^2). The cathode used was steel wire (10.0 × 0.3, length x diameter cm; geometric area = 9.56 cm^2). The wire electrodes were used in a spiral format. The exact inter-electrode gap between anode and cathode was 4 cm. The applied voltage was controlled by a Tensiometer, consisting in an adjustable DC Power Supply (HF-30035, Hikari, São Paulo, SP, Brazil), using 10 V constantly and evaluated the current created or 50 mA constant and the voltage created. Electrodes C modified (TiO_2@C) were prepared from carbon cylinder (C) anodes immersed in 1 mL solution of titanium (IV) isopropoxide and 3.5 mL of ethanol. A microwave-assisted deposition of titanium was carried out in a CEM-Discover model with PC control. Experiments were conducted in a closed vessel under continuous stirring and without cooling. Temperature, power irradiation and pressure were limited at 120 °C, 220 W and 220 PSI respectively [34–36].

AMX EO was conducted in a beaker at different times of reaction (typically 10, 20 and 40 min), with constant agitation and protected from light. At the end of each treatment time, the pH and temperature of the solution were determined by pHmeter (Quimis Scientific Apparatus, model Q488AS, Diadema, SP, Brazil). The assays were performed in duplicate.

2.3.1. Analysis AMX degradation

Degradation of AMX was monitored by UV-Vis spectrometry, differential pulse voltammetry (DPV) and mass spectrometry. The spectrophotometric measurements were conducted in UV-Visible Spectrophotometer (Quimis Scientific Apparatus, model Q798U2VS, Diadema, SP, Brazil) coupled to Unico Application Software (S2100 Series UV/Vis). Spectra were scanned from 200 to 1000 nm. To calculate the percentage of AMX removal (Equation (1)), the peak area obtained at 230 nm for AMX before and after treatments were calculated by the Origin® 8 software.

$$\% \text{ of AMX removal} = [(A_{pdrAMX} - A_{treatmentAMX}) / A_{pdrAMX}] \times 100 \quad (1)$$

Where A_{pdrAMX} stands for the peak area of AMX 12 mg/L and $A_{treatmentAMX}$ stands for the peak area of AMX after EO.

DPV measurements were performed with a potentiostat/galvanostat μAutolab III® integrated with GPES 4.9® software (Eco-Chemie, Utrecht, The Netherlands). For analysis were used electrochemical cell with 0.5 mL of sodium phosphate buffer 0.05 M (pH 8) and 0.5 mL of AMX solution before and after treatment, and three electrode systems: glassy carbon (working electrode), platinum wire (counter electrode) and Ag/AgCl/KCl 3 M (reference electrode) (purchased from Lab solutions, São Paulo, Brazil). The experimental conditions for DPV were: pulse amplitude 50 mV, pulse width 0.5 s and scan rate 10 mV s^{-1}, all performed at room temperature.

Mass spectrometry analysis was carried out in a mass spectrometer microTOF III (Brucker Daltonics, Bremen, Germany) equipped with a commercial ESI (Brucker Daltonics, Bremen, Germany). Analyses were performed by direct infusion (3 μL/min) after extraction in methanol and formic acid 1 mM. All analyses were performed in the positive mode. ESI (+) source conditions were as follows: nebulizer nitrogen gas with 200 °C temperature, 0.4 bar pressure; drying gas of 4 L/min; capillary voltage of −4 kV; transfer capillary temperature of 180 °C; end plate offset of −500 V; skimmer of 35 V and collision voltage of −1.5 V. Each spectrum was acquired using 2 microscans and processed with Data Analysis software (Brucker Daltonics, Bremen, Germany).

2.3.2. Fish Embryo Acute Toxicity (FET) tests

Fish Embryo Acute Toxicity (FET) test was carried out according to OECD guideline 236 [26] with some modifications. Twenty fertilized eggs per concentration were randomly selected and carefully distributed to a 96-well plate, filled with 200 μL of different EO of AMX with different electrolytes supported: 0.1 M of Na_2SO_4; 0.01, 0.1 M of NaCl, tap water or negative control (CN; maintenance water). Tests were performed in triplicate in a climate chamber at 26 ± 1 °C and 12 h under light. Embryo development was assessed at 24, 48, 72 and 96 h, using a stereomicroscope (BEL PHOTONICS STM 800).

2.4. Evaluation of antimicrobial activity

Broth microdilution was conducted to determine the minimum inhibitory concentration (MIC) of AMX before and after EO using TiO_2@C electrode with 0.01 M NaCl (10 min), according to the document "Performance Standards for Antimicrobial Susceptibility Testing" proposed by the Clinical and Laboratory Standards Institute [37]. The microorganisms used were *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922. Microdilution plates were incubated at 37 °C for 18–24 h and the results were observed with 0.5% solution of triphenyltetrazolium chloride.

2.5. Statistical analysis

GraphPad Prism® (version 5.0, GraphPad Software, San Diego, CA, USA) was used for the statistical analysis of FEET, biomarker determinations and FET test. Comparisons between different experimental exposure groups were performed with a one-way ANOVA followed by Dunnett's multiple comparison tests. Each experimental value was compared to its corresponding control. Statistical significance was accepted when the probability of the result assuming the null hypothesis (p) was less than 0.05.

3. Results

3.1. Fish embryo extended toxicity (FEET) test

The subchronic effects of different concentrations of AMX in embryo-larval stages of zebrafish were investigated during 7 dpf (or 168 h) of exposure. No significant lethal effect was observed during 168 h of exposure to AMX, as shown in Fig. 1A (24, 48 and 72 h: data not shown). Thus, it seems to have no embryotoxic potential in this tested condition; however, AMX significantly increased the body length of zebrafish larvae at 6.25, 12 and 25 mg/L (Fig. 1B).

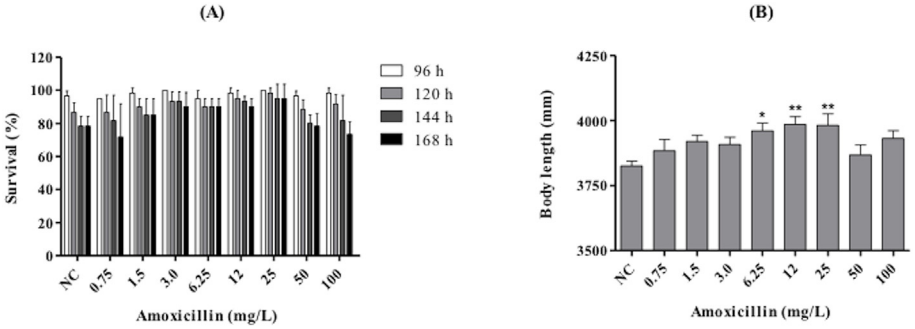

Fig. 1. (A) Survival rate of zebrafish life stages exposed to different AMX concentrations (mg/L) and analyzed at 96, 120, 144 and 168 h. (B) Effects of AMX on the body length of zebrafish larvae (μm) after 168 h of exposure. Bars represent mean ± SEM of three independent tests. *p < 0.05 and **p < 0.01 statistically different from the negative control (NC; reconstituted water).

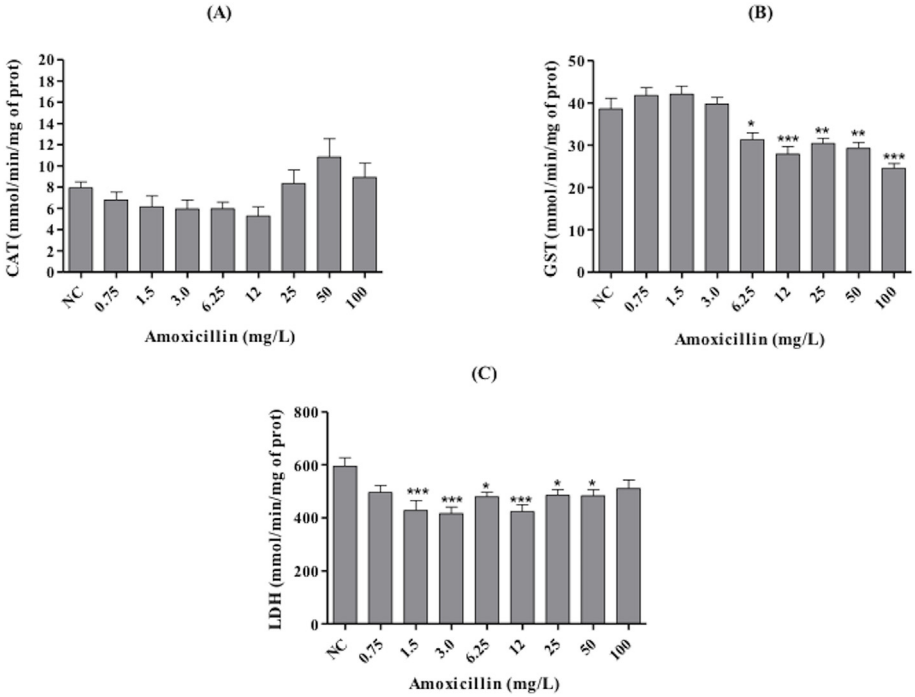

Fig. 2. Levels of (A) catalase (CAT), (B) glutathione S-transferase (GST) and (C) lactate dehydrogenase (LDH) activity in zebrafish larvae after 168 h of exposure to AMX expressed as mean values ± SEM. *p < 0.05, **p < 0.001 and ***p < 0.001 statistically different from the negative control (NC; reconstituted water).

3.1.1. Biochemical effects

The AMX effects on the biomarkers CAT, GST and LDH activities are presented in Fig. 2. Catalase (CAT) activity was not significantly altered in zebrafish embryos exposed to AMX (Fig. 2A). GST and LDH activity were significantly inhibited from 6.25 mg/L to 100 mg/L (Fig. 2B) and from 1.5 mg/L to 50 mg/L of AMX compared to control, respectively (Fig. 2C).

3.2. Electrochemical oxidation remediation of AMX

3.2.1. Effect of pH, temperature and voltage

The rate of chemical reactions is often and highly influenced by pH and temperature. In case of EO, the applied voltage has utmost importance, whereas pH and temperature can exert adverse effects over electrode polarization efficiency. The aforementioned parameters were verified in order to optimize the experimental conditions of anodic remediation and the achieved efficiency is presented in Fig. 3.

Temperature, pH and current effects before and after 10 min of treatment were observed, without any obvious changes that could suggest improvements in the selected EO remediation under the investigated conditions (Table 1). The current flow increase as the electrolyte concentration increased was expected, as it was not possible to measure the current with the equipment used for the medium with distilled water, since the concentration of salts were low.

AMX decay was monitored by means of UV-Visible Spectrophotometry. Initially (t = 0), two maximum absorption bands at 230 and 273 nm could be observed. AMX degradation after 40 min of EO in Na_2SO_4 was ca. 59% and 65% for C and TiO electrodes, respectively. Remarkably, AMX removal over 72% could be achieved in only 10 min in 0.1 M Na_2SO_4 using TiO_2@C as anode (Fig. 4 A). To simulate real situations, another supporting electrolyte (NaCl) and tap water were subjected to EO treatment for 10 min (Fig. 4 B). The highest efficiency for AMX removal was obtained with NaCl 0.1 M as supporting electrolyte. Remediation in tap water, despite not being most effective, could provide a maximum of 43% AMX removal in 10 min of reaction, which makes the study method potentially applicable for actual samples. In addition, a 68% removal was obtained after EO treatment in 0.01 M NaCl electrolyte (10 min).

3.2.2. Electrochemical behavior of AMX and remediated solutions

The effect of pH and ionic strength was also evaluated (Fig. 5A). A fresh AMX solution was compared to those subjected to EO for 40 min using C and Ti anodes, and 10 min using TiO_2@C (Fig. 5B). Interestingly, AMX solutions exhibited some peak shifts after treatment as compared to untreated solutions (0.63 V), being 0.68 V for Ti electrode, 0.75 V for C electrode and 0.77 V for TiO_2@C electrode (Fig. 5B). Results with TiO_2@C electrode in NaCl are shown in Fig. 5C.

3.2.3. Mass spectrometry analyses

Mass spectrometry analyses were further conducted to confirm results as the most sensitive technique for this purpose. The most abundant peak in the AMX untreated standard spectrum presented *m/z* of 388.0920, that could be assigned to sodium adduct $[M+Na]^+$. A lower intensity peak with *m/z* of 366.113 corresponded to $[M+H]^+$ (Supplement material 1).

3.2.4. Fish Embryo Acute Toxicity (FET) test

The acute effects of by-products generated after 10 min of AMX EO using TiO_2@C with different electrolytes (tap water; NaCl at 0.01 M and 0.1 M; Na_2SO_4 at 0.1 M) on zebrafish early-life stage were investigated during 96 h. No significant lethal effect was observed in treatments using electrolyte 0.01 M NaCl and tap water during 96 h, with survival rate ≥90% in all exposure periods, as shown in Fig. 6A. Comparably, by-products generated after EO in 0.1 M NaCl and Na_2SO_4 induced significant mortality in zebrafish larvae after 96 h of exposure (Fig. 6A). EO of AMX in 0.1 M NaCl significantly decreased the body length of zebrafish larvae after 96 h of exposure (Fig. 6B). In contrast, treatment using electrolyte 0.01 M NaCl and tap water did not alter the body length of larvae after 96 h of exposure (Fig. 6B).

Furthermore, no significantly malformations were observed after treatments with 0.01 M NaCl and tap water (data not shown). In contrast, by-products generated after EO with 0.1 M NaCl and Na_2SO_4 induced significant malformations in zebrafish after 48 h of exposure including: yolk sac edema (YE), blood cells accumulation (BA), delayed

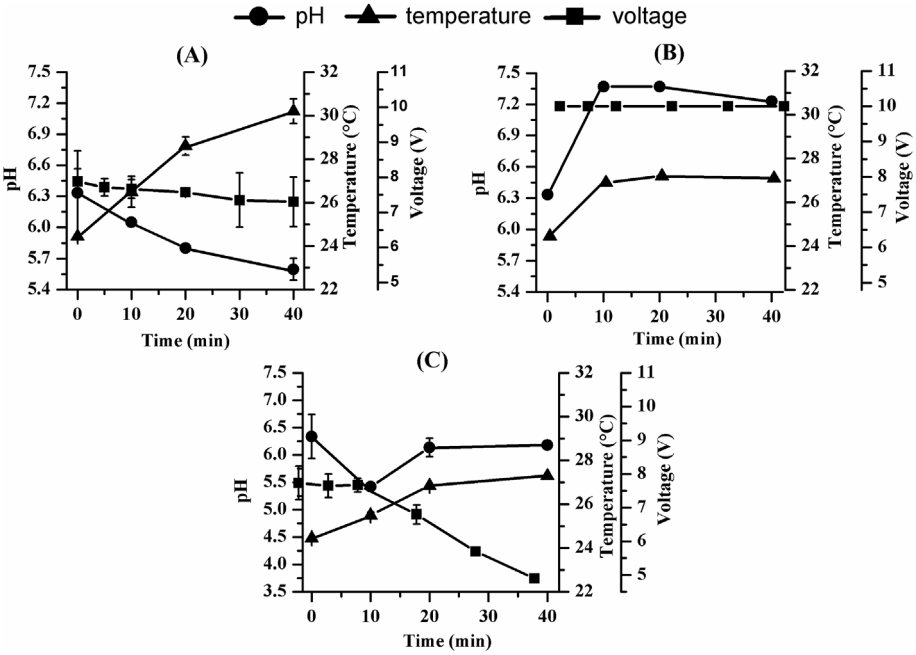

Fig. 3. Variation of pH, temperature and voltage during the EO of AMX in 0.1 M of Na_2SO_4 with anodes of carbon cylinder (A), titanium wire (B) and TiO_2@C (C).

Table 1
Variation of pH, temperature and current to AMX remediation after 10 min treatment with anode TiO_2@C using different electrolytes.

	pH		Temperature (°C)		Current (mA)[a]
	Initial	Final	Initial	Final	
Tap water	7.71	7.78 ± 0.057	27.60	27.90 ± 0.424	-[b]
0.01 M NaCl	8.31	8.77 ± 0.042	25.90	27.35 ± 0.495	20
0.1 M NaCl	7.08	8.20 ± 0.417	21.20	23.15 ± 0.495	60

[a] Voltage = 10 V.
[b] Below the capacity of the measuring equipment.

yolk sac absorption (YS) and pericardial edema (PE), with respect to control samples which presented a normal development (Table 2; Supplement material 2).

3.2.5. Antimicrobial activity

EO of AMX using TiO_2@C electrode with 0.01 M NaCl after 10 min (AMX-treated) was observed to significantly influence the antibiotic activity of AMX (Table 3).

In contact with *E. coli,* the concentration of AMX-treated capable of inhibiting microbial growth was about 250 times greater with respect to that required using AMX. The same fact occurred with *S. aureus*, since EO using TiO_2@C electrode with 0.01 M NaCl reduced the antimicrobial activity of AMX in ca.30 times. In tests using *P. aeruginosa* strain for both AMX and AMX-treated, MICs were higher than 1200 μg/mL. This response was expected for AMX before treatment, since *P. aeruginosa* is intrinsically resistant to most β-lactams [38] but were consequently inactive in the inhibition of bacterial growth after EO using TiO_2@C electrode with 0.01 M NaCl.

4. Discussion

In this study, lethal and sublethal effects of AMX before and after EO remediation using TiO_2@C electrode with different electrolytes (tap water; NaCl at 0.01 M and 0.1 M; Na_2SO_4 at 0.1 M) were reported using zebrafish early-life stage as an organism model. AMX did not induce significant mortality to zebrafish at concentrations ≤100 mg/L during extended exposure (7 dpf or 168 h; Fig. 1A). Oliveira et al. [7] evaluated the acute toxicity of AMX on embryos and larvae of zebrafish, and reported that AMX at concentrations ≤ 1125 mg/L induced no significant mortality in zebrafish after short-term exposure (96 h). In another study, Park and Choi [14], observed a LC_{50}-96 h greater than 1000 mg/L for *Oryzias latipes* fish exposed to AMX. As previously mentioned, data on potential effects of AMX on aquatic life are scarce in scientific literature, probably because in most cases, it has been associated with EC_{50} values > 1000 mg/L, indicating low toxicity

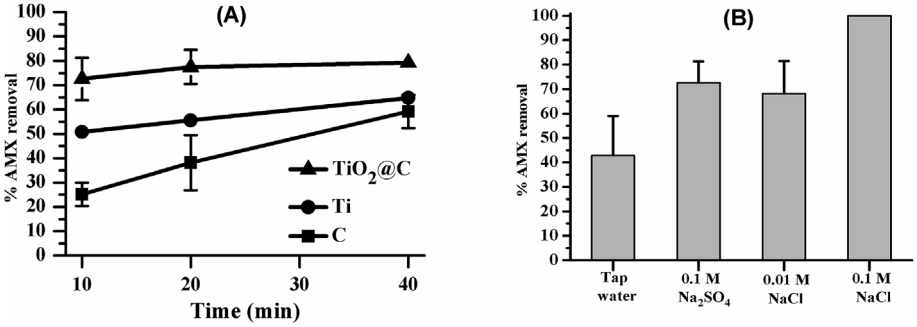

Fig. 4. (A) EO of AMX in 0.1 M Na_2SO_4 with different anodes. (B) Percentage of AMX removal after 10 min EO treatment with anode TiO_2@C using different supporting electrolysis.

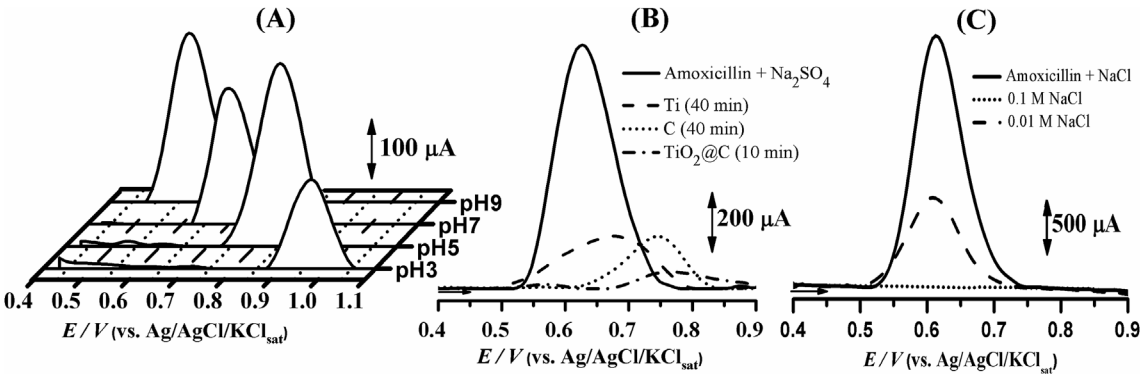

Fig. 5. (A) Differential pulse voltammograms obtained with vitreous carbon electrode in relation to optimum pH (B) AMX before and after EO on Na_2SO_4 (C) or NaCl support electrolytes.

[7,13,14].

In addition to low mortality, our study showed that AMX is able to increase body length of zebrafish larvae (6.25–25 mg/L) after 168 h of exposure (Fig. 1B). In fact, several antibiotics are used to stimulate fish growth [13,39,40] and enhance livestock feed efficiency [40,41], suggesting an improvement of nutrients absorption by weight gain. Until now, the mechanisms of growth promotion as a result of dietary antibiotic supplementation are still not exactly known [40]. Moreover, we evaluated biochemical responses of zebrafish larvae exposed to AMX through the analysis of biomarkers of oxidative stress (CAT and GST) and energetic metabolism (LDH). CAT activity was not significantly modified by AMX, while GST (6.25–100 mg/L) and LDH (1.5–50 mg/L) were inhibited (Fig. 2). Oliveira et al. [7] evaluated the effects of AMX (75, 128, 221 mg/L) on biomarkers activities in zebrafish larvae during 96 h and no changes in GST and LDH activities were detected.

Considering that GST is an enzyme that plays an important role in phase II of the detoxification process, responsible for the biotransformation of xenobiotic compounds and acts a marker of antioxidant mechanisms [42–45], our results showed that AMX inhibited the detoxification process and the antioxidant activity in the embryonic stage of zebrafish. A similar fact occurred with LDH, a key enzyme in the anaerobic pathway of energy production. LDH acts as a cell injury marker at tissues, particularly when high levels of energy are required in a short period of time as in case of chemical stress conditions [42,44–46]. In the present work, AMX significantly inhibited LDH, which may indicate a decreased glycolytic capacity in zebrafish larvae or suggest cellular necrosis leading to enzyme leakage and/or enzyme inhibition [45].

Considering theses toxic effects and the problem of antibiotic-resistance, we performed EO remediation of AMX. The assay solutions for EO, akin to the expected for aquatic systems, were not buffered. The water electrolysis in EO processes leads to electro generation of hydroxyl radical ($OH^{\bullet}$) and H^+, hence, causing pH changes, which support the efficiency and stability of anodic materials. Thus, pH was determined during treatment time. The final medium pH after 40 min of EO at C, Ti and TiO_2@C anodes were of 5.5, 7.3 and 6.2, respectively (Fig. 3).

Moreover, the exothermic behavior of water electrolysis was evidenced in all experimental conditions. The higher temperature increments observed for C anode can be attributed to ohmic resistance [47]. It was shown slightly variations of potential during the time of the treatment, however for the TiO_2@C anode there was a drop up to 3 V, indicating that the electrode might show less energy consumption over the remediation process (Fig. 3A e 3C).

The higher electroactive area of the modified anode, as well as the recognized electrocatalytic performance of TiO_2 nanostructured systems, have been proven with EO of AMX in this work. Supporting electrolytes, Na_2SO_4 and NaCl, were used to allow the flow of electric current and thus to promote AMX degradation. In addition, they also led to the formation of oxidants persulfate ($S_2O_8^{2-}$) and hypochlorite (ClO^-) ions, respectively, contributing to the AMX oxidation and its oxidation intermediates; therefore, increasing mineralization efficiency of the process [21,24,48].

The highest efficiency for removal of AMX was obtained with NaCl as supporting electrolyte. According to literature, NaCl is also the most efficient electrolyte in EO when compared to Na_2SO_4 [49,50]. While hydroxyl radicals are on the surface of the anode and have a very short lifetime, the active chlorine from NaCl may be present in the whole solution, since it does not necessarily remain on the surface of the anode and plays an important role as an organic oxidant [25].

AMX was also determined by means of DPV at different pH conditions. Fig. 5A shows a linear dependence between peak potential and pH, in agreement with the expected proton participation in the anodic reaction of AMX. The voltammetry analysis of selected assay solutions was performed in order to get new insights about the EO mechanism. Briefly, equal aliquots prior and after EO process were taken from assay solutions and determined by DPV at GCE. The peak potential, 1a, at E_{pa} = 0.63 V, corresponds to the EO of fresh AMX (Fig. 5B). The peak current falls intensely for treated samples, whereas akin to the observation previously in spectrometric determinations, the highest decay

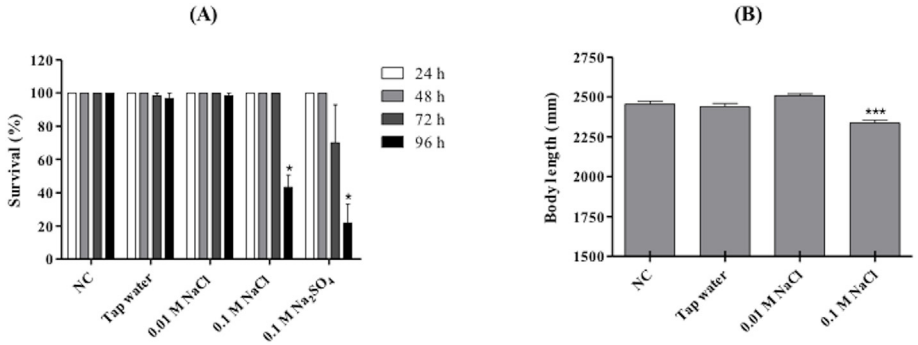

Fig. 6. (A) **S**urvival rate of zebrafish early-life stage exposed to EO of AMX using TiO_2@C electrode with different electrolytes (tap water; NaCl at 0.01 M and 0.1 M; Na_2SO_4 at 0.1 M) for 24, 48, 72 and 96 h. (B) Effects of different electrolytes used in EO of AMX on the body length of zebrafish larvae (µm) after 96 h of exposure. Bars represent mean values ± SEM of three tests. *p < 0.05 and ***p < 0.0001 statistically different from the respective negative control.

Table 2
Malformations rate in zebrafish early-life stage induced by EO of AMX using TiO_2@C electrode with 0.1 M Na_2SO_4 and 0.1 M NaCl after 48, 72, and 96 h of exposure expressed as mean values ± SEM of three independent tests. YE: yolk sac edema; BA: blood accumulation; PE: pericardial edema and YS: delayed yolk sac absorption.

Electrolytes	48 h		72 h		96 h	
	YE (%)	BA (%)	YS (%)	PE (%)	YS (%)	PE (%)
0.1 M Na_2SO_4	55 ± 0.15	55 ± 0.15	100 ± 0.00	76 ± 0.17	100 ± 0.00	82 ± 0.18
0.1 M NaCl	42 ± 0.16	72 ± 0.08	67 ± 0.47	50 ± 0.36	83 ± 0.24	69 ± 0.03

Table 3
Minimum inhibitory concentration of standard strains in contact with AMX before and after EO using TiO_2@C electrode with 0.01 M NaCl by 10 min.

Strain	Minimum inhibitory concentration (MIC) μg/mL	
	AMX	AMX-treated
Escherichia coli ATCC 25922	4.68	1200
Pseudomonas aeruginosa ATCC 27853	> 1200	> 1200
Staphylococcus aureus ATCC 25923	18.75	600

is achieved at TiO_2@C anode, even in a quarter of treatment time. Some peak shifts could be observed for AMX solutions after treatment (Fig. 5B). This is due to the final pH of treatments even using buffer to analyze solutions (it was not possible to maintain pH at 8 because the concentration of salts used as electrolytes was high enough to interfere in the buffering conditions).

Therefore, the difference of DPV and UV-visible in determination of AMX removal, which was 93% and 73%, respectively, for media containing 0.1 M Na_2SO_4 and TiO_2@C electrode can be explained by the final pH treatment. For electrolyte 0.01 M NaCl, the potential was the same before treatment, then results in DPV and Uv-Vis were similar, 65% and 68%, respectively (Figs. 4 and 5C).

Mass spectrometry analyses confirmed the most abundant peak $[M+Na]^+$ and a lower intensity peak $[M+H]^+$ in the AMX standard spectrum, in agreement with reported results by Frontistis et al. [22] (Supplement material 1). In addition, a typical fragment of electrochemistry AMX degradation (identified as I; identification error: −4.8) could be visualized in detailed mass spectrometry range 100–240 *m/z* for AMX treated solutions. Ganiyu et al. [23] reported the 167.02 *m/z* fragment among others after studying the kinetic mineralization and toxicity of amoxicillin.

As observed in other detection methods, UV-visible and DPV analyses, MS analysis proved that AMX removal was almost complete in only 10 min of treatment with TiO_2@C anode. So, we use this treatment with different electrolytes to evaluate the toxic potential for zebrafish early-life stages.

Considering AMX-treated, tap water removed 43% of AMX and it was non-toxic for zebrafish early-life stage. The highest efficiency for AMX removal (> 99%) occurred with 0.1 M NaCl; however, in this condition, by-products generated induced lethal (~57% of mortality; Fig. 6A) and sublethal (malformations) effects (Table 2 and Supplement material 2) and affected the body length of zebrafish larvae (Fig. 6B), whereas 0.01 M NaCl removed approximately 70% of AMX and caused no acute toxicity for zebrafish early-life stages (Fig. 6A).

The EO with 0.1 M Na_2SO_4 was responsible for 75% of AMX removal, but it induced the highest toxicity for zebrafish (Fig. 6A and supplement material 2), which can be related to intrinsic effects of Na_2SO_4. Ganiyu et al. [23] evaluated the potential of the substoichiometric titanium oxide (Ti_4O_7) as ceramic electrode for degradation and mineralization of aqueous solutions of 0.1 mM AMX containing 0.05 M Na_2SO_4 as supporting electrolyte. The potential toxicity of AMX during electrooxidation treatment was investigated by the bioluminescence inhibition assay (Microtox method), which is based on metabolic activity of the bacterial population (*Vibrio fischeri*) and any inhibition of enzymatic activity causes a corresponding decrease in bioluminescence. The authors observed an increased *V. fischeri* bioluminescence inhibition at the early stages of electrolysis reaching 100% of inhibition, due to formation of aromatic/cyclic organics as the predominant oxidation intermediates, which are more toxic than parent AMX solution. However, after 360 min of electrolysis, the bioluminescence inhibition decreased, indicating the mineralization/degradation of both AMX and its oxidation reaction intermediates into less toxic and biodegradable compounds [23].

Therefore, considering that EO of AMX using TiO_2@C electrode with 0.01 M NaCl was efficient in AMX removal and induced no toxic effect in zebrafish early-life stage, the antimicrobial potency of this antibiotic was evaluated through *broth microdilution technique*. Frontistis et al. [22] reported that AMX at 1455 μg/mL completely inhibited *Klebsiella pneumoniae* (NCTC 5056) and *Enterococcus faecalis* (ATCC 14506), but after EO treatment, no concentration of AMX-treated was able to completely inhibit growth of these bacteria, reducing only a rate of 50–60% of the bacterial population. In the present study, the antibiotic activity of AMX-treated was also observed at concentration ≥600 μg/mL (Table 3). There have been reports that AMX transformation by-products exhibit a small antimicrobial activity against some strains such as *S. aureus, E. faecalis* and *E. coli* [22,51,52]. It is important to report that, in this work, AMX and its by-products are in much higher concentrations than those found in effluents, surface waters and groundwater. Based on that, it can be suggested that these by-products do not present antimicrobial activity in levels found in the environment.

5. Conclusion

In summary, our findings demonstrated that AMX induced no mortality but affected the zebrafish development (increased body length) and the activity of enzymatic biomarkers such as GST and LDH during extended exposure. The EO remediation was efficient in the AMX degradation and can be applied for other organic pollutants. TiO_2@C anode achieved a complete AMX removal in just a few minutes and the efficiency of the supported electrolytes occurred in the following order: 0.1 M NaCl > Na_2SO_4 > 0.01 M NaCl > tap water. The potential toxicity order to zebrafish early life-stage considering the lethal and sublethal effects was as follows: Na_2SO_4 > 0.1 M NaCl > 0.01 M NaCl = tap water. Additionally, EO of AMX using TiO_2@C electrode with 0.01 M NaCl was able to inhibit the antimicrobial activity of AMX, reducing the possibility of developing bacterial resistance. Therefore, EO using TiO_2@C electrode could be an interesting alternative for drinking water and wastewater treatment plants, considering its low cost and environmental safety.

Conflicts of interest

The authors declare there were no conflicting interests.

Acknowledgements

The authors are grateful to the Goiás Research Foundation (FAPEG), Funding Authority for Studies and Projects (FINEP - Process No. 01.14.0140.00) and to the National Council for Scientific and Technological Development (CNPq: INCT/DATREM - Process No. 465571/2014-0), for their financial support and scholarships. The authors would like also to thank the Faculty of Pharmacy - Federal University of Goiás and Biological Sciences Institute - University of Brasília for their help in carrying out this research.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.cbi.2018.06.017.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cbi.2018.06.017.

References

[1] M. Llorca, M. Farré, E. Eljarrat, S. Díaz-Cruz, S. Rodríguez-Mozaz, D. Wunderlin, D. Barcelo, Review of emerging contaminants in aquatic biota from Latin America: 2002-2016, Environ. Toxicol. Chem. 36 (2016) 1716–1727, http://dx.doi.org/10.1002/etc.3626.

[2] I. Xagoraraki, D. Kuo, Water pollution: emerging contaminants associated with drinking water, Int. J. Environ. Res. Publ. Health 6 (2008) 539–550, http://dx.doi.org/10.1016/B978-012373960-5.00292-6.

[3] F.O. Agunbiade, B. Moodley, Pharmaceutical as emerging organic contaminants in Umgeni River water system, KwaZulu-Natal, South Africa, Environ. Monit. Assess. 186 (2014) 7273–7291, http://dx.doi.org/10.1007/s10661-014-3926-z.

[4] A.B.A. Boxall, L.A. Fogg, P. Kay, P.A. Blackwel, E.J. Pemberton, A. Croxford, Prioritisation of veterinary medicines in the UK environment, Toxicol. Lett. 142 (2003) 207–218, http://dx.doi.org/10.1016/S0378-4274(03)00067-5.

[5] P. Bottoni, S. Caroli, A.B. Caracciolo, Pharmaceuticals as priority water contaminants, Toxicol. Environ. Chem. 92 (2010) 549–565, http://dx.doi.org/10.1080/02772241003614320.

[6] C. Song, C. Zhang, L. Fan, L. Qiu, W. Wu, S. Meng, G. Hu, B. Kamira, J. Chen, Occurrence of antibiotics and their impacts to primary productivity in fishponds around Tai Lake, China, Chemosphere 161 (2016) 127–135, http://dx.doi.org/10.1016/j.chemosphere.2016.07.009.

[7] R. Oliveira, S. McDonough, J.C.L. Ladewig, A.M.V.M. Soares, A.J.A. Nogueira, I. Domingues, Effects of oxytetracycline and amoxicillin on development and biomarkers activities of zebrafish (*Danio rerio*), Environ. Toxicol. Pharmacol. 36 (2013) 903–912, http://dx.doi.org/10.1016/j.etap.2013.07.019.

[8] M. González-Pleiter, S. Gonzalo, I. Rodea-Palomares, F. Leganés, R. Rosal, K. Boltes, E. Marco, F. Fernández-Piñas, Toxicity of five antibiotics and their mixtures towards photosynthetic aquatic organisms: implications for environmental risk assessment, Water Res. 47 (2013) 2050–2064.

[9] Y. Zhong, Z.F. Chen, X. Dai, S.S. Liu, G. Zheng, X. Zhu, S. Liu, Y. Yin, G. Liu, Z. Cai, Investigation of the interaction between the fate of antibiotics in aquafarms and their level in the environment, J. Environ. Manag. 207 (2018) 219–229.

[10] M. Pan, L.M. Chu, Occurrence of antibiotics and antibiotic resistance genes in soils from wastewater irrigation areas in the Pearl River Delta region, southern China, Sci, Total Environ 624 (2018) 145–152, http://dx.doi.org/10.1016/j.scitotenv.2017.12.008.

[11] J.L. Magalhães, S. Borschiver, Amoxicillin and Ampicillin: import trends and increasing use in Brazil, Chim. Oggi-Chem. Today 30 (2012) 91–93.

[12] A. Pérez-Parada, A. Agüera, M.M. Gómez-Ramos, J.F. García-Reyes, H. Heinzen, A.R. Fernández-Alba, Behavior of amoxicillin in wastewater and river water: identification of its main transformation products by liquid chromatography/electrospray quadrupole time-of-flight mass spectrometry, Rapid Commun, Mass Spectrom. 25 (2011) 731–742, http://dx.doi.org/10.1002/rcm.4902.

[13] C. Carballeira, M.R. Orte, I.G. Viana, T.A. DelValls, A. Carballeira, Assessing the toxicity of chemical compounds associated with land-based marine fish farms: the sea urchin embryo bioassay with *Paracentrotus lividus* and *Arbacia lixula*, Arch. Environ. Contam. Toxicol. 63 (2012) 249–261.

[14] S. Park, K. Choi, Hazard assessment of commonly used agricultural antibiotics on aquatic ecosystems, Ecotoxicology 17 (2008) 526–538, http://dx.doi.org/10.1007/s10646-008-0209-x.

[15] T.B. Minh, H.W. Leung, I.H. Loi, W.H. Chan, M.K. So, J.Q. Mao, D. Choi, J.C.W. Lam, G. Zheng, M. Martin, J.H.W. Lee, P.K.S. Lam, B.J. Richardson, Antibiotics in the Hong Kong metropolitan area: ubiquitous distribution and fate in victoria harbour, marine poll, Bulletin 58 (2009) 1052–1062, http://dx.doi.org/10.1016/j.marpolbul.2009.02.004.

[16] A.J. Watkinson, E.J. Murby, D.W. Kolpin, S.D. Costanzo, The occurrence of antibiotics in an urban watershed: from wastewater to drinking water, Sci. Total Environ. 407 (2009) 2711–2723, http://dx.doi.org/10.1016/j.scitotenv.2008.11.059.

[17] J. Yin, Z. Meng, M. Du, C. Liu, M. Song, H. Wang, Pseudo-template molecularly imprinted polymer for selective screening of trace ß-lactam antibiotics in river and tap water, J. Chromatogr. A 1217 (2010) 5420–5426, http://dx.doi.org/10.1016/j.chroma.2010.06.044.

[18] M.A.F. Locatelli, F.F. Sodré, W.F. Jardim, Determination of antibiotics in brazilian surface waters using liquid chromatography–electrospray tandem mass spectrometry, Arch. Environ. Contam. Toxicol. 60 (2011) 385–393, http://dx.doi.org/10.1007/s00244-010-9550-1.

[19] I. Sirés, E. Brillas, Remediation of water pollution caused by pharmaceutical residues based on electrochemical separation and degradation technologies: a review, Environ. Int. 40 (2012) 212–229, http://dx.doi.org/10.1016/j.envint.2011.07.012.

[20] B.G. Padilla-Robles, A. Alonso, S.A. Martínez-Delgadillo, M. González-Brambila, U.J. Jaúregui-Haza, J. Ramírez-Muñoz, Electrochemical degradation of amoxicillin in aqueous media, Chem. Eng. Process 94 (2015) 93–98, http://dx.doi.org/10.1016/j.cep.2014.12.007.

[21] 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, http://dx.doi.org/10.1016/j.cej.2014.09.100.

[22] Z. Frontistis, M. Antonopoulou, D. Venieri, I. Konstantinou, D. Mantzavinos, Boron-doped diamond oxidation of amoxicillin pharmaceutical formulation: statistical evaluation of operating parameters, reaction pathways and antibacterial activit, J. Environ. Manag. (2016) 1–10, http://dx.doi.org/10.1016/j.jenvman.2016.04.035.

[23] S.O. Ganiyu, N. Oturan, S. Raffy, M. Cretin, R. Esmilaire, E. van Hullebusch, G. Esposito, M.A. Oturan, Sub-stoichiometric titanium oxide (Ti_4O_7) as a suitable ceramic anode for electrooxidation of organic pollutants: a case study of kinetics, mineralization and toxicity assessment of amoxicillin, Water Res. 106 (2016) 171–182, http://dx.doi.org/10.1016/j.watres.2016.09.056.

[24] F.C. Moreira, R.A.R. Boaventura, E. Brillas, V.J.P. Vilar, Electrochemical advanced oxidation processes: a review on their application to synthetic and real wastewaters, Appl. Catal. B Environ. 202 (2017) 217–261, http://dx.doi.org/10.1016/j.apcatb.2016.08.037.

[25] Y. Juang, E. Nurhayati, C. Huang, J.R. Pan, S. Huang, A hybrid electrochemical advanced oxidation/microfiltration system using BDD/Ti anode for acid yellow 36 dye wastewater treatment, Separ. Purif. Technol. 120 (2013) 289–295, http://dx.doi.org/10.1016/j.seppur.2013.09.042.

[26] OECD, Test No. 236: Fish Embryo Acute Toxicity (FET) Test, OECD Publishing, 2013.

[27] C.B. Kimmel, W.W. Ballard, S.R. Kimmel, B. Ullman, T.F. Schilling, Stages of embryonic development of the zebrafish, Dev. Dynam. 203 (1995) 253–310.

[28] A. Clairborne, Catalase activity, in: G. RA (Ed.), CRC Handbook of Methods in Oxygen Radical Research, CRC Press, 1985, pp. 283–284.

[29] W.H. Habig, W.B. Jakoby, Assays for differentiation of glutathione S-transferases, Meth. Enzymol. 77 (1981) 398–405.

[30] M.F. Frasco, L. Guilhermino, Effects of dimethoate and beta-naphthoflavone on selected biomarkers of *Poecilia reticulate*, Fish Physiol. Biochem. 26 (2002) 149–156.

[31] A. Vassault, Lactate Dehydrogenase. Methods of Enzymatic Analysis, Enzymes: Oxireductases, Transferase, Academic Press, 1983, pp. 118–126.

[32] T.C. Diamantino, E. Almeida, A.M.V.M. Soares, L. Guilhermino, Lactate dehydrogenase activity as an effect criterion in toxicity tests with *Daphnia magna* straus, Chemosphere 45 (2001) 553–560.

[33] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1976) 248–254.

[34] J.M. Campelo, T.D. Conesa, M.J. Gracia, M.J. Jurado, R. Luque, J.M. Marinas, A.A. Romero, Microwave facile preparation of highly active and dispersed SBA-12 supported metal nanoparticles, Green Chem. 10 (2008) 853–858, http://dx.doi.org/10.1039/B801754A.

[35] A.M. Balu, D. Dallinger, D. Obermayer, J.M. Campelo, A.A. Romero, D. Carmona, F. Balas, K. Yohida, P.L. Gai, C. Vargas, C.O. Kappe, R. Luque, Insights into the microwave-assisted preparation of supported iron oxide nanoparticles on silica-type mesoporous materials, Green Chem. 14 (2012) 393–402, http://dx.doi.org/10.1039/c1gc16119a.

[36] G.S. Lobón, A. Yepez, L.F. Garcia, R.L. Morais, B.G. Vaz, V.V. Carvalho, G.A.R. Oliveira, R. Luque, E.S. Gil, Efficient electrochemical remediation of microcystin-LR in tap water using designer TiO_2@carbon electrodes, Sci. Rep. 7 (2017) 41326, http://dx.doi.org/10.1038/srep41326.

[37] Clinical and Laboratory Standards Institute. M100-s27 Performance Standards for Antimicrobial Susceptibility Testing, (2017).

[38] Y. Morita, J. Tomida, Y. Kawamura, Responses of *Pseudomonas aeruginosa* to antimicrobials, Front. Microbiol. 4 (2014) 422, http://dx.doi.org/10.3389/fmicb.2013.00422.

[39] R. Andreozzi, V. Caprio, C. Ciniglia, M. Champdoré, R. Giudice, R. Marotta, E. Zuccato, Antibiotics in the environment: occurrence in Italian STPs, fate, and preliminary assessment on algal toxicity of amoxicillin, Environ. Sci. Technol. 38 (2004) 6832–6838, http://dx.doi.org/10.1021/es049509a.

[40] R.M. Reda, R.E. Ibrahim, E.N.G. Ahmed, Z.M. El-Bouhy, Effect of oxytetracycline and florfenicol as growth promoters on the health status of cultured *Oreochromis niloticus*, Egypt. J. Aquat. Res. 39 (2013) 241–248, http://dx.doi.org/10.1016/j.ejar.2013.12.001.

[41] H.Y. Done, A.K. Venkatesan, R.U. Halden, Does the recent growth of aquaculture create antibiotic resistance threats different from those associated with land animal production in agriculture? AAPS J. 17 (2015) 513–524, http://dx.doi.org/10.1208/

s12248-015-9722-z.
[42] T.S. Andrade, J.F. Henriques, A.R. Almeida, A.L. Machado, O. Koba, P.T. Giang, A.M.V.M. Soares, I. Domingues, Carbendazim exposure induces developmental, biochemical and behavioural disturbance in zebrafish embryos, Aquat. Toxicol. 170 (2016) 390–399, http://dx.doi.org/10.1016/j.aquatox.2015.11.017.
[43] F.R. Abe, C. Gravato, A.M.V.M. Soares, D.P. Oliveira, Biochemical approaches to assess oxidative stress induced by exposure to natural and synthetic dyes in early life stages in zebrafish, J. Toxicol. Environ. Health 80 (2017) 1259–1268.
[44] C.L.G. Rivero-Wendt, R. Oliveira, M.S. Monteiro, I. Domingues, A.M.V.M. Soares, C.K. Grisolia, Steroid androgen 17α-methyltestosterone induces malformationsand biochemical alterations in zebrafish embryos, Environ. Toxicol. Pharmacol. 44 (2016) 107–113, http://dx.doi.org/10.1016/j.etap.2016.04.014.
[45] C. Quintaneiro, D. Patrício, S.C. Novais, A.M.V.M. Soares, M.S. Monteiro, Endocrine and physiological effects of linuron and S-metolachlor in zebrafish developing embryos, Sci. Total Environ. (2016), http://dx.doi.org/10.1016/j.scitotenv.2016.11.153.
[46] S. Coelho, R. Oliveira, S. Pereira, C. Musso, I. Domingues, R.C. Bhujel, A.M.V.M. Soares, A.J.A. Nogueira, Assessing lethal and sub-lethal effects of trichlorfon on different trophic level, Aquat. Toxicol. 103 (2011) 191–198, http://dx.doi.org/10.1016/j.aquatox.2011.03.003.
[47] R.A. Baricatti, C. Silva, M.L. Souza, C.A. Lindino, M.F. Rosa, Degradação hidrolítica e fotoquímica da amoxicilina na presença de β-ciclodextrina, Ecl. Quimera 33 (2008) 79–84, http://dx.doi.org/10.1590/S0100-46702008000400010.
[48] A. Thiam, M. Zhou, E. Brillas, I. Sirés, Two-step mineralization of Tartrazine solutions: study of parameters and by-products during the coupling of electrocoagulation with electrochemical advanced oxidation processes, Appl. Catal. B Environ. 150 (2014) 116–125, http://dx.doi.org/10.1016/j.apcatb.2013.12.011.
[49] C. Carvalho, A. Fernandes, A. Lopes, H. Pinheiro, I. Gonçalves, Electrochemical degradation applied to the metabolites of Acid Orange 7 anaerobic biotreatment, Chemosphere 67 (2007) 1316–1324.
[50] P.K. Brahman, R.A. Dar, K.S. Pitre, Conducting polymer film based electrochemical sensor for the determination of amoxicillin in micellar media, Sensor. Actuator. B. 176 (2013) 307–314, http://dx.doi.org/10.1016/j.snb.2012.09.007.
[51] D. Dimitrakopoulou, I. Rethemiotaki, Z. Frontistis, N.P. Xekoukoulotakis, D. Venieri, D. Mantzavinos, Degradation, mineralization and antibiotic inactivation of amoxicillin by UV-A/TiO2 photocatalysis, J. Environ. Manag. 98 (2012) 168–174, http://dx.doi.org/10.1016/j.jenvman.2012.01.010.
[52] Y.J. Jung, W.G. Kim, Y. Yoon, J.W. Kang, Y.M. Hong, H.W. Kim, Removal of amoxicillin by UV and UV/H_2O_2 processes, Sci. Total Environ. 420 (2012) 160–167, http://dx.doi.org/10.1016/j.scitotenv.2011.12.011.