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High frequency ultrasound as a selective advanced oxidation process to remove penicillinic antibiotics and eliminate its antimicrobial activity from water

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ABSTRACT

This work studies the sonochemical degradation of a penicillinic antibiotic (oxacillin) in simulated pharmaceutical wastewater. High frequency ultrasound was applied to water containing the antibiotic combined with mannitol or calcium carbonate. In the presence of additives, oxacillin was efficiently removed through sonochemical action. For comparative purposes, the photo-Fenton, TiO_2 photocatalysis and electrochemical oxidation processes were also tested. Therefore, the evolution of the antibiotic and its associated antimicrobial activity (AA) were monitored. A high inhibition was found for the other three oxidation processes in the elimination of the antimicrobial activity caused by the additives; while for the ultrasonic treatment, a negligible effect was observed. The sonochemical process was able to completely degrade the antibiotic, generating solutions without AA. In fact, the elimination of antimicrobial activity showed an excellent performance adjusted to exponential kinetic-type decay. The main sonogenerated organic by-products were determined by means of HPLC-MS. Four intermediaries were identified and they have modified the penicillinic structure, which is the moiety responsible for the antimicrobial activity. Additionally, the possible oxacillin sonodegradation mechanism was proposed based on the evolution of the by-products and their chemical structure. Furthermore, the high-frequency ultrasound action over 120 min readily removed oxacillin and eliminated its antimicrobial activity. However, the pollutant was not mineralized even after a long period of ultrasonic irradiation (360 min). Interestingly, the previously sonicated water containing oxacillin and both additives was completely mineralized using non-adapted microorganisms from a municipal wastewater treatment plant. These results show that the sonochemical treatment transformed the initial pollutant into substances that are biotreatable with a typical aerobic biological system.

1. Introduction

Currently, it is common to find diverse pharmaceutical products in the effluents of municipal wastewater plants [1,2]. Many of these substances are recalcitrant to conventional treatment systems and, consequently, they make their way to the natural environment. Pharmaceuticals in natural water can interact with wild organisms which could lead to DNA mutations, alterations of the cellular development and even cause death. Particularly, antibiotic pollution can induce microorganism population selection and generate microbial resistance [2]. Therefore, resistant genes in environmental bacteria can be transferred to humans and animals, increasing the risk and diminishing our ability to treat illnesses [1].

Penicillinic antibiotics are used against several infections and are recognized among the most consumed antibiotics in the world [2]. Oxacillin is an example of such penicillinic antibiotics. It is widely used in clinic treatments against aerobic gram-positive cocci [3]. Oxacillin has been detected in effluents of wastewater plants and in natural water bodies [4]. Currently, the treatment of illness associated to oxacillin-resistant microorganisms represents a serious problem in some hospitals around the world [5]. One of the main sources of antibiotics in wastewater is the pharmaceutical industry [2]. Inefficient or inexistent sewage treat-

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ments in plants and factories allow active substances to make their way to municipal wastewater treatment plants and, subsequently, to natural water bodies. Thus, studies on how to eliminate this problematic compound by means of effective processes have become necessary.

In recent years, the sonochemical treatment (an advanced oxidation process, AOP) has shown to be efficient to remediate water polluted with pharmaceutical compounds [6–8]. Sonochemistry is based on a cyclical sequence where micro-bubbles form and grow until reaching a critical size; then, they collapse violently in a process called acoustic cavitation, which is induced by the interaction between ultrasonic waves and dissolved gases in aqueous solutions. The collapse of the micro-bubbles generates small hot spots with singular conditions of pressure (~1000 atm) and temperature (~5000 K) [9]. Under such conditions, hydroxyl radicals are generated by the dissociation of water molecules and oxygen (Eqs. (1)–(4)). By means of the recombination of these radicals, hydrogen peroxide can also be formed (Eq. (5)).

$$H_2O+))) \longrightarrow \cdot H + \cdot OH \quad (1)$$

$$O_2+))) \longrightarrow 2\ \cdot O \quad (2)$$

$$H_2O + \cdot O \longrightarrow 2\ \cdot OH \quad (3)$$

$$O_2 + \cdot H \longrightarrow \cdot O + \cdot OH \quad (4)$$

$$2 \cdot OH \longrightarrow H_2O_2 \quad (5)$$

The application of high frequency ultrasound to eliminate the antimicrobial activity from synthetic pharmaceutical wastewater containing the oxacillin antibiotic (OXA) was tested in this study. As active ingredients are commonly mixed with other compounds [10], special attention was paid to other substances that could also be present in pharmaceutical wastewater. Therefore, this research work was focused on studying the effects on the efficiency of the sonochemical process of a common excipient (mannitol, MAN) used in the preparation of antibiotics and other typical active ingredient (calcium carbonate, CC) [11]. The hydrogen peroxide accumulation rates during the sonochemical process were determined in order to understand the action of the additives on the elimination of both OXA and AA. The antimicrobial activity elimination kinetic constants for different water samples were obtained and used to quantitatively compare the efficiency of the sonochemical process with the efficiency of the photo-Fenton, TiO_2 photocatalysis and anodic oxidation processes. Additionally, the main sonogenerated intermediaries were determined by means of HPLC-MS, and their evolution under ultrasonic treatment was monitored. Finally, the bio-treatability of previously-sonicated water containing OXA and additives was assessed by means of the application of an aerobic biological process using non-adapted microorganisms.

2. Materials and methods

2.1. Reagents

Oxacillin was provided by Sigma–Aldrich. Formic acid, hydrogen peroxide and calcium carbonate were purchased from Carlo-Erba. Mannitol, sulfuric acid, methanol, acetonitrile, sodium chloride, sodium phosphate, sodium hydroxide, sodium meta-bisulfite, potassium iodide, ammonium heptamolybdate, heptahydrate ferrous sulfate and nutrient agar were provided by Merck. Potato dextrose agar was provided by Oxoid. Titanium dioxide P-25 was supplied by Evonik (Degussa). Due to the fact that the antibiotic is found in pharmaceutical wastewater in concentrations of mg L^{-1} [12,13] and the commercial formulations could contain additives ten times more concentrated than the active ingredient, the OXA and the additives (calcium carbonate and mannitol) were used at concentrations of 47.23 μmol L^{-1} (20 mg L^{-1}) and 472.3 μmol L^{-1}, respectively. All solutions were prepared with distilled water and the processes were carried out at and initial pH of 5.6 (which is the natural pH of the OXA solution).

2.2. Reaction systems

Sonochemical experiments were carried out in a cylindrical glass reactor containing 250 mL of the solution, using a homemade ultrasound generator (Fig. SM 1, in Supplementary Material). Ultrasonic waves of 275 kHz (at 60 W) were emitted from the bottom of the reactor by a piezo-electric disk (with a 4 cm diameter) fixed on a Pyrex plate (with a 5 cm diameter). It has been reported that a high production of hydroxyl radical occurs in the range of 200–300 kHz [14]. Therefore, 275 kHz of frequency was used in all of the experimental tests. The power dissipated by the reactor was 20.7 W (34.5% of the electrical power input) calculated by the calorimetric method [15]. The reactor was kept at 20 ± 1 °C using a Bioblock Scientific cryothermostat (Huber).

The biological process was carried out using aerobic microorganisms from the San Fernando municipal wastewater treatment plant (Medellín, Colombia): 25 mL of inoculum were added to 250 mL of solution in order to biologically treat the water. This system was aerated with a Resun aquarium pump (AC-9904). It was then stirred using a VELP BOD shaker and the samples were treated at environmental temperature (27 ± 0.5 °C). Before applying the biological process, the pH of the solutions was adjusted to 6.5 with sodium hydroxide, and the residual hydrogen peroxide was eliminated using sodium meta-bisulfite.

Electrochemical oxidation experiments were conducted in an electrolytic cell (Fig. SM 2) containing 150 mL of OXA solution under constant stirring conditions. Degradation experiments were conducted at constant current density (5 mA cm^{-2}), and using a Ti/IrO_2 anode with 4 cm^2 of working surface area. The cathode was a zirconium spiral electrode of 10 cm^2. 0.065 mol L^{-1} NaCl was used as the supporting electrolyte. For TiO_2 photocatalysis and photo-Fenton process was used a homemade aluminum reflective reactor (Fig. SM 3), equipped with five 30 W Philips lamps (TL-D Actinic BL). 150 W of light power was applied to 100 mL of OXA solution placed in beakers with constant stirring. In the photo-Fenton process, 1000 μmol L^{-1} and 90 μmol L^{-1} of H_2O_2 and Fe (II) respectively were used. Before the analyses, the residual H_2O_2 also was eliminated using sodium meta-bisulfite. In the heterogeneous photocatalysis, 0.50 g L^{-1} of TiO_2 was used. The lamps were turned on after the adsorption equilibrium was achieved under constant stirring (30 min). Before analyzing the treated samples, the catalyst was separated by centrifugation for 15 min at 3200 rev min^{-1} in a centrifuge (Centaur 2), and filtered using a cellulose mesh of 0.45 μm (Advantech).

2.3. Analyses

A quantitative analysis of OXA was carried out using a HPLC Waters (486) instrument with a C-18 column (Merck LiChrospher) and a UV detector set at 225 nm. The injection volume was 40 μL. The mobile phase was a phosphate buffer (0.02 mmol L^{-1}, pH 5)/acetonitrile/methanol, 64/27/9 (% v/v) in isocratic mode (0.4 mL min^{-1}). The oxidants generated during the process were determined through iodometry [16].

The antimicrobial activity (AA) was determined by analyzing the inhibition zone in the agar diffusion test, using *Staphylococcus aureus* ATCC 6538 as the indicator microorganism. 30 μL of the sample solution were seeded on Petri dishes containing 5 mL of