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Use of sunlight to degrade oxytetracycline in marine aquaculture's waters☆

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ABSTRACT

Oxytracycline (OTC) is a broad spectrum antibiotic authorized for use in European aquaculture. Its photo-degradation has been widely studied in synthetic aqueous solutions, sometimes resorting to expensive methods and without proven effectiveness in natural waters. Thus, this work studied the possibility to apply the solar photo-degradation for removal of OTC from marine aquaculture's waters. For that, water samples were collected at different locals of the water treatment circuit, from two different aquaculture companies. Water samples were firstly characterized regarding to pH, salinity, total suspended solids (TSS), organic carbon and UV–Vis spectroscopic characteristics. Then, the samples were spiked with OTC and irradiated using simulated sunlight in order to evaluate the matrix effects on OTC photo-degradation. From kinetic results, the apparent quantum yields and the outdoor half-life times, at 40°N for midsummer and midwinter days were estimated by the first time for these conditions. For a midsummer day, at sea level, the outdoor half-life time predicted for OTC in these aquaculture's waters ranged between 21 and 25 min. Additionally, the pH and salinity effects on the OTC photo-degradation were evaluated and it has been shown that high pH values and the presence of sea salt increase the OTC photo-degradation rate in aquaculture's waters, compared to results in deionised water. The results are very promising to apply this low-cost methodology using the natural sunlight in aquaculture's waters to remove OTC.

1. Introduction

Aquaculture has become the fastest growing food production sector and may be practiced in extensive, semi-intensive or intensive system. One of the main characteristics that distinguish the types of production is its density. The density of production generally ranges between 0.02 and 0.5 tonnes/ha/year in an extensive aquaculture system, while in an intensive aquaculture system the values may vary between 5 and 100 tonnes/ha/year (Lucas and Southgate, 2012). Aquaculture has gained prominence due to the stabilization of the fish captures and the increase of fish consumption in the last years (FAO, 2014). However, the higher the production intensity greater the tendency to accumulate particles, fat and other compounds present in the food or excreted by the fish that may propitiate the appearance of diseases. As result, sometimes it is necessary to resort to the use of therapeutic agents such as disinfectants or antibiotics to prevent or treat diseases. The classes of antibiotics authorized for oral use in fish in Europe are tetracyclines, trimethoprim, sulphonamides, quinolones, β-lactams, fluoroquinolones and fenicols (EMA, 1999; FAO, 2005). Among them, oxytetracycline (OTC), which belongs to the tetracyclines class, is one of the antibiotics most frequently used in aquaculture (Rigos and Troisi, 2005). It is a broad spectrum antibiotic incorporated in the feed and adopted for treatment and control of a wide variety of bacterial infections. The doses of its administration are dependent on clinical and physiological conditions of fish. For example, a daily dose of 55 mg of OTC per Kg of live weight is recommended for application to species such as *Oncorhynchus* sp, *Sparus aurata* or *Psetta máxima* (Cenavisa, 2016). OTC excretions occur mainly through the urine and faeces as unchanged form since OTC undergoes minimal or no metabolism (EMA, 1995). For example, Rigos et al. (2004)measured OTC in faecal excretions and estimated that significant quantities of un-metabolized OTC can be passed unabsorbed through the body of treated sparids and

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be excreted into the local marine environment (Rigos et al., 2004).

Photo-degradation of OTC under UV or visible light has been studied by several authors (Jiao et al., 2008; Lopez-Penalver et al., 2010; Xuan et al., 2010; Pereira et al., 2013; Zhao et al., 2013) and proposed to remove OTC from surface and ground-waters (Lopez-Penalver et al., 2010). Most of these studies use UV with TiO_2, which is a procedure that, besides requiring considerable energetic costs, has been demonstrated to loss efficiency in the presence of chloride ions (Pereira et al., 2013) and thus is less adequate for salt waters. Contrarily, the main advantages of the photo-degradation using sunlight are the natural source of light and the low cost, comparatively with UV radiation. There already are some studies that report to photo-degradation of OTC using simulated or natural sunlight (Lunestad et al., 1995; Pouliquen et al., 2007; Chen et al., 2008; Xuan et al., 2010; Leal et al., 2015). Among them, only Lunestad et al. (1995) and Pouliquen et al. (2007) used natural and artificial saltwater, respectively. However, the kinetics and mechanisms of contaminants photo-degradation may be dependent on water composition, namely on inorganic compounds and type and content of dissolved organic matter (DOM) (Prabhakaran et al., 2009). In what concerns DOM, its influence is dependent on its composition and concentration (Guerard et al., 2009; Leal et al., 2015), which can vary among different coastal waters and which can be influenced by the fish production procedures in aquaculture. Besides, the composition and concentration of DOM may also vary along water treatment circuit of aquaculture systems. Among the several inorganic compounds present in aquaculture's water, the presence of calcium and magnesium have been proposed as affecting the OTC photo-degradation (Xuan et al., 2010; Chen et al., 2011). In fact, these are two of the main constituents of seawater (salinity ≈ 35‰). For example, Wright et al. (2013) found concentrations of 1290 mg/L for magnesium and 412 mg/L for calcium, whereby their possible influence on OTC photo-degradation should not be neglected (Wright et al., 2013).

Thus, in this work, the possibility of applying solar photo-degradation for removal of OTC (Fig. S1, in supplementary information – SI) in marine aquaculture's waters was studied. The aquaculture's water samples, collected at specific points of the water treatment from two aquaculture companies, were firstly characterized (physical-chemical characterization). Secondly, those water samples were used to perform the OTC photo-degradation experiments and evaluate the matrix effects on OTC photo-degradation. By the first time, the apparent quantum yields of photoreactions, as well as the outdoor half-life times of OTC in marine aquaculture's waters, at latitude 40°N and for different seasons are estimated. In the present work, the effects of pH and salinity on OTC photo-degradation kinetics were also studied in order to explain their contribution in these aquatic systems.

2. Material and methods

2.1. Chemicals

Oxytetracycline hydrochloride (Table S1, in SI) was provided from Sigma Aldrich. The stock solution of phosphate buffer 0.1 M was prepared in milli-Q water (1 L) from the mixture of 0.05 mol of sodium dihydrogen phosphate dehydrate – $NaH_2PO_4 \cdot 2H_2O$ (Fluka, Biochemika, ultra ≥99%) and 0.05 mol of di-sodium hydrogen phosphate dihydrate – $Na_2HPO_4 \cdot 2H_2O$ (Fluka, Biochemika) for each litre of buffer. The pH of solution was adjusted to 7.3 with a NaOH solution. To prepare the synthetic saltwater solutions (21‰), "Tropic Marin Pro Reef Sea Salt" (Tropical Marine Centre Limited-TMC) was dissolved in phosphate buffer solution (0.001 M) and the pH was also adjusted to 7.3. Tropic Marin Pro Reef Sea Salt is free from synthetic additives and contains no nitrates, phosphates or silicates (MarineDepot, 2015). The aquaculture's water samples were collected from two different companies of fish production in brackish water.

2.2. Sampling and characterization of aquaculture's waters

Water samples from two different aquaculture companies in Portugal (designated as company A and company B) were collected twice (two independent samplings per each company). The first and second sampling events were in April/May 2014 and in September/October 2014, respectively. These two companies have distinct water circuits but have some common steps of water treatment. In company A, the water from the fish tanks is submitted to mechanical filtration and then to ozonation, while in company B, there is a biological treatment between mechanical filtration and ozonation. Company A has not biological treatment. In both companies, brackish underground water is captured through a water bore. Water samples were collected after mechanical filter (L1) and after ozonation (L2) in companies A and B, and also after the biological treatment (L1b) in company B. For each sampling event, three to six replicates were collected at each sampling site. All water samples collected were analysed by UV–Vis and characterized regarding to the following parameters: pH, salinity, total suspended solids (TSS), dissolved organic carbon (DOC), total and inorganic carbon of suspended particles (TC_{part} and IC_{part}), dissolved calcium and dissolved magnesium. The main equipments used were a T90 + UV/VIS Spectrophotometer (PG Instruments Ltd), with a slit width of 2 nm, and quartz cuvettes of 1 cm path length; a Total Organic Carbon Analyser (Shimadzu) – liquid and solid sample modules: TOC-VcPH and SSM-5000A, respectively; a pH meter (Eutech Instruments, EcoScan) and a salinity meter (WTW-Cond 330i). An atomic absorption spectrophotometer Perkin Elmer, Analyst 100 was used to determine calcium and magnesium by flame atomic absorption spectroscopy (air-acetylene). The calibration details of methods are presented in the Supplementary information (SI). The water samples were filtered using a glass microfiber filter of 2 μM (47 mm, GF/C Whatman®) and the filters were dried at 105 °C in order to determine TSS. For the determination of dissolved organic carbon (DOC, mg C/L) the filtered water samples were previously acidified with 2% (v/v) of HCl 2 M and purged with nitrogen. TC_{part} and IC_{part} were quantified analysing each glass microfiber filter containing the suspended solids of each water sample. In the case of IC_{part}, 4 mL of phosphoric acid (one part of undiluted acid 85% to two parts of water) were previously added before heating the sample to 200 °C. To determine the dissolved calcium and magnesium the water samples were filtered, using a membrane filter (0.2 μm, NL16 Whatman), and acidified with HNO_3 65% to pH ≤ 2. The characterization of water samples was performed according to the recommendations of Standard Methods For the Examination of Water & Wastewater (Eaton et al., 2005).

2.3. Photo-degradation experiments

Irradiation experiments were performed with aqueous solutions of OTC 4 mg/L (8×10^{-6} mol L^{-1}). The water samples collected at the different sampling sites above mentioned were spiked with OTC 4 mg/L and submitted to artificial solar irradiation. Photo-degradation experiments were performed with filtered and non-filtered samples. Solutions of OTC 4 mg/L in phosphate buffer 0.001 M at different salinities (0 and 21‰) were also prepared and irradiated for comparison. All irradiations were achieved in a sunlight simulator (Solarbox 1500 – Co.fo.me.gra, Italy) using an irradiance of 55 W/m^2 (290–400 nm), corresponding to 550 W/m^2 in all spectral range. This sunlight simulator is equipped with a