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Reduction of clarithromycin and sulfamethoxazole-resistant *Enterococcus* by pilot-scale solar-driven Fenton oxidation

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HIGHLIGHTS

- Solar Fenton oxidation of an antibiotic mixture in three water matrices was studied.
- The disinfection of enterococci and removal of antibiotic resistance was examined.
- There is higher antibiotic resistance prevalence to sulfamethoxazole than clarithromycin.
- Enterococci resistance to a mixture is less prevalent than to one antibiotic.
- The final solar Fenton effluent toxicity is low in real and simulated effluents.

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ABSTRACT

The presence of pathogenic antibiotic-resistant bacteria in aquatic environments has become a health threat in the last few years. Their presence has increased due to the presence of antibiotics in wastewater effluents, which are not efficiently removed by conventional wastewater treatments. As a result there is a need to study the possible ways of removal of the mixtures of antibiotics present in wastewater effluents and the antibiotic-resistant bacteria, which may also spread the antibiotic resistance genes to other bacterial populations. In this study the degradation of a mixture of antibiotics i.e. sulfamethoxazole and clarithromycin, the disinfection of total enterococci and the removal of those resistant to: a) sulfamethoxazole, b) clarithromycin and c) to both antibiotics have been examined, along with the toxicity of the whole effluent mixture after treatment to the luminescent aquatic bacterium *Vibrio fischeri*. Solar Fenton treatment (natural solar driven oxidation) using Fenton reagent doses of 50 mg L^{-1} of hydrogen peroxide and 5 mg L^{-1} of Fe^{3+} in a pilot-scale compound parabolic collector plant was used to examine the disinfection and antibiotic resistance removal efficiency in different aqueous matrices, namely distilled water, simulated and real wastewater effluents. There was a faster complete removal of enterococci and of antibiotics in all aqueous matrices by applying solar Fenton when compared to photolytic treatment of the matrices. Sulfamethoxazole was more efficiently degraded than clarithromycin in all three aqueous matrices (95% removal of sulfamethoxazole and 70% removal of clarithromycin in real wastewater). The antibiotic resistance of enterococci towards both antibiotics exhibited a 5-log reduction with solar Fenton in real wastewater effluent. Also after solar Fenton treatment, there were 10 times more antibiotic-resistant enterococci in the presence of sulfamethoxazole than in the presence of clarithromycin. Finally, the toxicity of the treated wastewater to *V. fischeri* remained very low throughout the treatment time.

1. Introduction

Antibiotics are the most frequently consumed pharmaceutical compounds since they are used in human and veterinary medicine to treat bacterial infections (Kemper, 2008; Gardner et al., 2011). Because of their incomplete biological metabolism, the residues of these compounds are excreted in their original form and/or as metabolites, ending up in sewage treatment plants. Due to their antibacterial nature, they are not completely treatable in the biological wastewater treatment and as a result they commonly turn up in the aquatic environment.

The release of antibiotics into the aquatic environment encourages the presence of antibiotic-resistant pathogenic and non-pathogenic bacteria, which pose a serious risk to human health (Baquero et al., 2008). The aquatic environment comprises a medium of spread of

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these microorganisms, some of which are resistant to various currently used antibiotics, and an environment in which resistance genes can form reservoirs of antibiotic resistance (Biyela and Bezuidenhout, 2004). These genetic elements can be horizontally transferred between donor and receptor cells of the same or different taxonomic categories, or vertically to the next generation (Schwartz et al., 2006).

The aquatic bacterial communities contain important human pathogens, including the gram-positive indicator microorganisms *Enterococcus* spp. enterococci, which pose a threat to health as they are opportunistic pathogens, being often associated with nosocomial infections (Klein, 2003; Łuczkiewicz et al., 2010). The ability of these communities to gain antibiotic-resistant genes has enabled them to spread the acquired antibiotic resistance to other bacteria (Da Silva et al., 2005).

The ability of natural sunlight to disinfect water is known as solar photo-inactivation (Fisher et al., 2012). Studies conducted on various pathogenic microorganisms using disinfection with natural sunlight have demonstrated the ability of specific solar wavelengths to inactivate faecal pathogens (Wegelin et al., 1994; Sinton et al., 2002; Boehm et al., 2009).

Studies have demonstrated the presence of various antibiotics in surface waters and wastewater effluents. Their concentrations in the environment are typically very low, in the ng L^{-1} to μg L^{-1} range (Fatta-Kassinos et al., 2011; Verlicchi et al., 2012; Michael et al., 2013). Despite their low environmental concentrations, their persistence in aquatic resources may pose acute or chronic risks for aquatic organisms and humans (Lindqvist et al., 2005; Kümmerer, 2009).

Clarithromycin (CLA) is a macrolide antibiotic with a lactone function (Calza et al., 2012), and it is commonly used for bacteriostatic treatment of upper and lower respiratory tract infections, skin and mycobacterial infections. The presence of CLA in wastewater effluents has been reported in various studies (Yasojima et al., 2006; Lin et al., 2009; Loganathan et al., 2009; Gros et al., 2010; Jelic et al., 2011; Martinez Bueno et al., 2012). Its concentration in wastewater effluents varies in different studies, from 150 to 460 ng L^{-1} worldwide (Al Aukidy et al., 2012). Gros et al. (2010) and De la Cruz et al. (2012) demonstrated the presence of CLA in 100% of the treated samples examined, showing no removal efficiency during conventional wastewater treatment. CLA removal has been associated to sorption onto solid sludge particles during wastewater treatment by more than 20% according to Jelic et al. (2011), while increased microorganism presence in the water matrix has been shown to enhance its biodegradability, as the production of enzymes by microorganisms may enhance the removal of antibiotics during treatment (Ghosh et al., 2008). Furthermore, the stability of the antibiotic and the production of metabolites and other derivatives during biological treatment are important, as they may be as active as the parent compound or even toxic to organisms, something that stands true for all pharmaceutical compounds. Very few studies examine the impact of CLA in wastewater effluents regarding its ability to be toxic to organisms and to produce antibiotic resistance in microorganisms once it is released into the environment (Harada et al., 2008; Kim et al., 2009).

Sulfamethoxazole (SMX) is a widely used synthetic sulfonamide antibiotic used to treat urinary infections (Dodd and Huang, 2004). Its concentration in wastewater effluents varies worldwide, from 3 to 840 ng L^{-1} (Hirsch et al., 1999; Andreozzi et al., 2003; Bueno et al., 2007; Al Aukidy et al., 2012). The efficiency of the conventional wastewater treatment process in removing SMX has been widely investigated. According to Bendz et al. (2005), less than 70% SMX removal is achieved by the conventional wastewater treatment. SMX removal has also been examined with solar Fenton oxidation. Trovó et al. (2009) investigated its removal at a bench and pilot plant scale using photocatalytic treatment. The results indicated a strong influence of the water matrix on the degradation of the antibiotic, while an increase in hydrogen peroxide (H_2O_2) reduced the toxicity of the treated samples. Various studies have investigated the antibiotic resistance caused by SMX in wastewater effluents (Schwartz et al., 2003; Reinthaler et al., 2003; Ferreira Da Silva et al., 2005), while other studies have examined its toxicity (González et al., 2007; Quinn et al., 2008; Trovó et al., 2009). No studies have been made though to examine both the antibiotic resistance patterns and the toxicity of effluents containing SMX and CLA, together in mixture.

Photo-Fenton oxidation is an attractive option among the various existing AOPs, for the treatment of wastewater contaminated with antibiotics, microorganisms and other biological material not efficiently removed by conventional biological treatment methods (Esplugas et al., 2007; Klavarioti et al., 2009). It has low cost, provides a homogeneous treatment system (Will et al., 2004), very low reagent toxicity and it is simple in application (Li et al., 2012). Also, the use of simple reactors along with the possibility of the use of natural sunlight (i.e. solar Fenton) as the radiation source resulting in a reduction of the energy demand, has given the process more attention as an attractive wastewater treatment method (Badawy et al., 2009).

The addition of H_2O_2 is common for disinfection purposes in agricultural water (Liberti and Notarnicola, 1999). H_2O_2 can be directly toxic to bacteria and may lead to their inactivation, or cause a shock response to *Enterococcus faecalis* populations, making them less resistant to the presence of antibiotics and other toxic substances, as well as disinfection treatments such as solar Fenton (Koivunen and Heinonen-Tanski, 2005). A delay in disinfection may be attributed to an adaptation of the bacteria to the new dominant environmental conditions, making them increasingly resistant to the applied photo-Fenton treatment due to the development of self-defence and repair mechanisms against the treatment (Pericone et al., 2003). As living cells are exposed to H_2O_2 through their metabolic processes at steady concentrations, they have mechanisms to remove it with the utilisation of specific intracellular antioxidants (Cadenas and Davies, 2000).

Few studies have been published so far examining the effect of pilot-scale photocatalytic treatments on the disinfection of antibiotic-resistant bacteria in a mixture of antibiotics in aqueous matrices. The antibiotic resistance of pathogenic indicator bacteria such as enterococci to a single antibiotic has been examined without looking at the prevalence of antibiotic resistance in the presence of a mixture of antibiotics so far (Ferreira da Silva et al., 2005; Michael et al., 2012; Rizzo et al., 2013). As a result, the purpose of this work was to investigate the following aspects which have not previously been studied: the disinfection potential of pilot-scale solar Fenton oxidation and the removal of antibiotic-resistant bacteria in the presence of a mixture of antibiotics, all of which were examined in three aqueous matrices namely distilled water, simulated secondary wastewater and real wastewater. Moreover, the degradation of the selected antibiotic mixture i.e. SMX and CLA was evaluated. Finally the toxicity of the final treated effluent was assessed using the luminescent aquatic bacterium *Vibrio fischeri*. To the authors' knowledge this work is the first to provide information on all of these aspects of solar Fenton treatment.

2. Materials and methods

2.1. Chemicals and reagents

The standards of the antibiotic compounds were of high purity and were not purified further (CLA ≥98% and SMX ≥98%, both Fluka Analytical). The reagents used in the solar Fenton experiments were $Fe(NO_3)_3 \cdot 9H_2O$ (Merck) and H_2O_2 (35% w/w, Scharlau). The pH was adjusted with 2 N H_2SO_4 around 4, followed by stirring to eliminate carbonates and bicarbonates. For toxicity and other bioassays, the residual H_2O_2 of the treated solution was removed with bovine liver catalase (Sigma Aldrich) after neutralisation by 2 N NaOH (Pancreac). Titanium (IV) oxysulphate solution (Fluka Analytical) was used for the determination of H_2O_2, whereas acetic acid, 1–10 phenanthroline chloride monohydrate and ascorbic acid were used for the determination of total iron residues.