Journal of Environmental Management 198 (2017) 256–265

Contents lists available at ScienceDirect

Journal of Environmental Management

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

Research article

Electrochemical advanced oxidation processes for *Staphylococcus aureus* disinfection in municipal WWTP effluents

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ARTICLE INFO

Article history:
Received 21 December 2016
Accepted 23 April 2017
Available online 2 May 2017

Keywords:
Staphylococcus aureus
Disinfection
Photo-electro-Fenton
Gas diffusion electrode

ABSTRACT

This paper presents the *Staphylococcus aureus* inactivation in a simulated wastewater treatment plant effluent by different electrochemical techniques, including the photo-electro-Fenton process. *S. aureus*, dissolved organic carbon (DOC), total oxidants and H_2O_2 concentrations, as well as pH, were monitored during the assays. An electrolytic cell, including a UVA lamp, a gas diffusion electrode (GDE) as cathode and an IrO_2 anode, was used to conduct the experiments under galvanostatic conditions (20 mA). Low inactivation (−0.4) and low DOC removal were achieved within 120 min when applying the GDE-IrO_2 system, in which bacteria disinfection was caused by the generated H_2O_2. When light was combined with GDE-IrO_2, the process efficiency noticeably increased (−3.7 log inactivation) due to the synergistic effect between UVA and H_2O_2. Introducing iron (5 mg L^{-1} Fe^{2+}) into the system also produced higher disinfection and DOC mineralization. The electro-Fenton process (GDE-IrO_2+Fe^{2+}) led to a bacterial reduction of −0.9 log units and DOC reduction of 14%, while with the photo-electro-Fenton process (GDE-IrO_2+UVA + Fe^{2+}) −5.2 units of bacteria and 26% of DOC were removed. Increasing the current intensity (20 mA, 30 mA and 40 mA) in the photo-electro-Fenton system increased H_2O_2 production and, consequently, augmented the bacterial inactivation (−5.2 log, −6.2 log and −6.5 log, respectively). However, mineralization extent slightly increased or remained practically the same. When comparing the influence of Fe^{2+} and Fe^{3+} on photo-electro-Fenton, similar *S. aureus* inactivation was observed, while DOC removal was higher with Fe^{2+} (31%) than with Fe^{3+} (19%). Finally, by testing the system with a Ti anode, the direct anodic oxidation contribution of the IrO_2 anode was identified as negligible.

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1. Introduction

Staphylococcus aureus is a facultative anaerobic, Gram-positive, coccoid bacterium, which has the capacity to colonize almost every tissue of the human body, causing different diseases, such as wound infections, septicaemia or endocarditis (Haley and Skaar, 2012). Since microbial removal is not the objective of wastewater treatment plants (WWTPs), *S. aureus*, along with other pathogenic microbes, can be found in municipal effluents in relatively high concentrations (Mosteo et al., 2013). Moreover, antibiotic-resistant pathogens and their genes (for instance, methicillin-resistant and vancomycin-resistant *S. aureus*) can be easily spread through wastewaters (Mandal et al., 2015; Wan and Chou, 2014). For these reasons, WWTPs effluents pose a potential health problem, especially when reused (Rosenberg-Goldstein et al., 2014). In order to avoid this risk, there are several international guidelines (e.g. USEPA, 2012; WHO, 2006) and laws (e.g. Spanish Royal Decree 1620/2007) which establish quality criteria for wastewater reuse depending of its final application (municipal, industrial, agricultural, recreational or environmental). Therefore, WWTPs effluents may have to undergo additional disinfection treatments for a safe reuse.

Chlorination, ozonation and UVC light are among the conventional disinfection techniques most widely used for water treatment. As these technologies imply some disadvantages, advanced oxidation processes (AOPs), such as TiO_2 photocatalysis or the

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http://dx.doi.org/10.1016/j.jenvman.2017.04.070
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Fenton process, have gained importance during the last decades (Malato et al., 2009; Moncayo-Lasso et al., 2008). AOPs are based on the generation of reactive oxygen species (ROS), including the hydroxyl radical •OH, which is a non-selective and very powerful oxidizing agent (Parsons, 2004). Besides, ROS are not only able to inactivate microorganisms but also to degrade organic and inorganic substances.

The Fenton reaction consists in the activation of hydrogen peroxide by ferrous iron salts (Fenton, 1894) and involves the production of ROS (Haber and Weiss, 1934) (Eqs. (1) and (2)), being the rate limiting step the regeneration of ferric ion into ferrous ion (Malato et al., 2009) (Eqs. (3) and (4)):

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^- \quad (1)$$

$$H_2O_2 + {}^{\bullet}OH \rightarrow H_2O + HO_2{}^{\bullet} \quad (2)$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HO_2{}^{\bullet} + H^+ \quad (3)$$

$$Fe^{3+} + HO_2{}^{\bullet} \rightarrow Fe^{2+} + O_2 + H^+ \quad (4)$$

This problem is overcome in the presence of light ($h\nu$), where the ferric ion, which can be found in water as an iron hydroxide ($Fe(OH)^{2+}$), can be reduced again to the ferrous form (Eq. (5)). This is known as the photo-Fenton process (Zepp et al., 1992).

$$Fe(OH)^{2+} + h\nu \rightarrow Fe^{2+} + {}^{\bullet}OH \quad (5)$$

When the H_2O_2 is continuously electro-generated *in-situ*, along with the addition of an iron catalyst, it is called the electro-Fenton (EF) process. One of the main advantages of electro-Fenton over the conventional Fenton process is that it avoids the problems associated with hydrogen peroxide management. For instance, H_2O_2 production at industrial scale is carried out by the anthraquinone oxidation process, which requires great energy consumption and generates waste. Moreover, the transport, storage and handling of H_2O_2 involve important risks as well (Campos-Martin et al., 2006).

Introducing light into the EF system leads to the photo-electro-Fenton (PEF) process. The electrochemical advanced oxidation processes (EF and PEF) use electrolytic cells in which the cathode is fed with oxygen or air to produce the hydrogen peroxide. Graphite, carbon felt or gas diffusion electrodes (GDE) are commonly employed as cathodes while for anode materials graphite, Pt, metal oxides and BDD are usually preferred (Brillas et al., 2009).

The electrochemical advanced oxidation processes (EAOPs) have been widely studied for the removal of organic compounds from water. Degradation of pesticides (Abdessalem et al., 2010; Iglesias et al., 2015), antibiotics (Annabi et al., 2016; Özcan et al., 2016; Sopaj et al., 2016), dyes (Bedolla-Guzman et al., 2016; García-Rodríguez et al., 2016) and mineralization of TOC from wastewaters (Ren et al., 2016) by EF is frequently found in literature. However, only a few publications on the use of EF for water disinfection are available. Da Pozzo et al. (2008) proved the efficiency of electro-generated H_2O_2 combined with iron sulfate to disinfect seawater. Aziz et al. (2013) applied for the first time the electro-Fenton process for removing coliform bacteria from landfill leachates. Recently, Cotillas et al. (2015) used Fe anodes and carbon felt cathodes to promote the Fenton reaction for the inactivation of *Escherichia coli* present in a real WWTP effluent. However, in spite of the aforementioned current high risk associated to the presence of *Staphylococcus aureus* in WWTPs and the good performance showed by the EAOPs, the inactivation of this microorganism using these technologies has not been reported.

The aim of this research was to assess the efficiency of the photo-electro-Fenton process on the inactivation of *Staphylococcus aureus* in a simulated municipal wastewater treatment plant effluent. An electrolytic cell provided with a GDE cathode, an IrO_2 anode and a UV lamp was used for the experiments. The influence of each reaction system (photolysis (UV light), electrochemical disinfection (electro-generated H_2O_2) and electro-Fenton (electro-generated $H_2O_2 + Fe^{2+}$)) was studied. Moreover, different variables of the photo-electro-Fenton process were analysed, such as the current intensity, type of iron and type of anode. Finally, for further comparison, the energy consumption of each photo-electro-Fenton treatment was determined.

2. Experimental

2.1. Chemicals

$NaHCO_3$, NaCl, $CaSO_4{\cdot}2H_2O$, $MgSO_4$, KCl, K_2HPO_4, $CaCl_2{\cdot}2H_2O$, $MgSO_4{\cdot}7H_2O$, KI, $[NH_4]_6Mo_7O_{24}{\cdot}4H_2O$, NH_4VO_3, $FeSO_4{\cdot}7H_2O$, $Fe_2(SO_4)_3{\cdot}9H_2O$ and plate count agar were provided by Merck. Urea was supplied by M&B Laboratory Chemicals. Peptone and meat extract were provided by Oxoid. All solutions and samples were prepared using milli-Q water.

2.2. Water composition

The sample consisted of simulated municipal wastewater treatment plant (WWTP) effluent with the following composition [1, 2]: $NaHCO_3$ (96 mg L^{-1}), NaCl (7 mg L^{-1}), $CaSO_4{\cdot}2H_2O$ (60 mg L^{-1}), urea (6 mg L^{-1}), $MgSO_4$ (60 mg L^{-1}), KCl (4 mg L^{-1}), K_2HPO_4 (0.28 mg L^{-1}), $CaCl_2{\cdot}2H_2O$ (4 mg L^{-1}), peptone (32 mg L^{-1}), $MgSO_4{\cdot}7H_2O$ (2 mg L^{-1}) and meat extract (22 mg L^{-1}). $MgSO_4{\cdot}7H_2O$ 0.05 M was added as the supporting electrolyte to avoid an excessive voltage increase and consequent anode damage during the electrochemical processes. The sample was sterilized and initial pH was adjusted to 8 with H_2SO_4. An inoculum of *Staphylococcus aureus* was daily prepared by incubation of a stock culture of pure *S. aureus* cells in Luria Bertani (LB) broth for 3–4 h at 37 °C with continuous shaking. The inoculum (1 mL) diluted 1:100 in sterilized NaCl 0.9% was added to 250 mL of sample previous to each assay, leading to a concentration of $\approx 10^6$ CFU 100 mL^{-1}.

2.3. Reaction systems

The following treatments were applied: photolysis (UV), electrochemical disinfection (GDE + IrO_2), photo-electrochemical disinfection (UV + GDE + IrO_2), electro-Fenton (GDE + IrO_2+Fe^{2+}) and photo-electro-Fenton (UV + GDE + IrO_2 or Ti + $Fe^{2+/3+}$).

All the experiments were conducted in a glass reactor wrapped with aluminium foil used as electrolytic cell containing 250 mL of sample. The cathode was a gas diffusion electrode (GDE) consisting of a 5 cm^2 graphite membrane through which a constant flow of air was supplied, thus providing oxygen and continuous stirring to the reactor. The anode was made of IrO_2 and had 4 cm^2 of working surface area. The electrochemical treatments were carried out applying a current intensity of 20 mA under galvanostatic conditions. A UVA lamp of 368 nm, located inside the electrolytic cell, was used in the photo-treatments. In the Fenton processes, 5 mg L^{-1} of Fe^{2+} were added as $FeSO_4{\cdot}7H_2O$. Additionally, some factors were studied for the photo-electro-Fenton process: current intensity (20, 30 and 40 mA), iron type (Fe^{2+} or Fe^{3+}-added as $Fe_2(SO_4)_3{\cdot}9H_2O$) and anode type (IrO_2 or Ti).

Parallel to each assay, an *S. aureus* survival control was carried out with 250 mL of sample contained in a glass beaker with constant stirring under laboratory light conditions. No mortality of the bacteria was observed in these controls.

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