Available online at www.sciencedirect.com

ScienceDirect

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

Disinfection of urban wastewater by solar driven and UV lamp – TiO_2 photocatalysis: Effect on a multi drug resistant *Escherichia coli* strain

L. Rizzo [a,*], A. Della Sala [a], A. Fiorentino [a], G. Li Puma [b]

[a] Department of Civil Engineering, University of Salerno, via Giovanni Paolo II, 1, 84084 Fisciano, SA, Italy
[b] Environmental Nanocatalysis & Photoreaction Engineering, Department of Chemical Engineering, Loughborough University, Loughborough LE11 3TU, United Kingdom

ARTICLE INFO

Article history:
Received 7 August 2013
Received in revised form
8 January 2014
Accepted 11 January 2014
Available online 24 January 2014

Keywords:
Antibiotic resistant bacteria
Indigenous *E. coli* strain
Kirby–Bauer method
Radiation absorption-scattering modeling
Solar photocatalysis

ABSTRACT

The effect of TiO_2 photocatalysis on the inactivation of an antibiotic resistant *Escherichia coli* strain selected from an urban wastewater treatment plant (UWWTP) effluent was investigated. Different light sources including a 250 W wide spectrum lamp, a 125 W UV-A lamp and solar radiation, as well as, photocatalysts loadings (TiO_2 Degussa P25) in the range from 0.05 to 2.00 g TiO_2 L^{-1} were evaluated. The higher efficiency (total bacterial inactivation after 10 min of irradiation) was observed in the absence of TiO_2 when the wastewater was irradiated using the 250 W lamp. In the presence of TiO_2 a decreasing inactivation trend was observed (99.76% and 72.22% inactivation after 10 min irradiation at 0.10 and 2.00 g TiO_2 L^{-1} respectively). Under solar simulated conditions the highest inactivation efficiency (93.17%) after 10 min of irradiation was achieved at the lower photocatalyst loading (0.05 g TiO_2 L^{-1}). The concept of "reactor optical thickness" was introduced to explain the rates of disinfection observed. The optimum photocatalyst loading estimated by radiation absorption-scattering modeling was found to be 0.1 g TiO_2 L^{-1} for all lamps. The difference between experimental tests and modeling may be due to TiO_2 particles aggregation. Comparative kinetic tests between solar and solar simulated photocatalytic (SSP) processes using 0.05 g TiO_2 L^{-1} in suspension showed a quite similar inactivation behavior up to 30 min of irradiation, but only the SSP process resulted in a total inactivation of bacteria after 60 min of exposure. Antibiotic resistant test (Kirby–Bauer) on survived colonies showed that the SSP and SP processes affected in different ways the resistance of *E. coli* strain to the target antibiotics.

1. Introduction

The widespread use of antibiotics for human, veterinary and aquaculture purposes results in their continuous release into the environment (Díaz-Cruz et al., 2003; Batt et al., 2006; Watkinson et al., 2009; Fram and Belitz, 2011) since these species are only partially metabolized by organisms. Their occurrence into the environment is of particular concern because of the development of antibiotic resistance in

* *Corresponding author*. Tel.: +39 (0)89 969334; fax: +39 (0)89 969620.
E-mail address: l.rizzo@unisa.it (L. Rizzo).

0043-1354/$ – see front matter
http://dx.doi.org/10.1016/j.watres.2014.01.020

bacterial populations (ARB), which results in the loss of antibiotics effectiveness for the treatment of several diseases (Schwartz et al., 2003). There are four main genetic reactors in which antibiotic resistance evolves (Baquero et al., 2008): (i) human and animal microbiota, (ii) hospitals, long-term care facilities, farms, or any other place in which susceptible individuals are crowded and exposed to bacterial exchange, (iii) wastewater facilities and (iv) soil, surface and ground water, where the bacterial organisms originated in the previous reactors, mix and counteract with environmental organisms. Among wastewater facilities, conventional urban wastewater treatment plants (UWWTPs) typically operated by biological processes, are suspected to contribute to ARB selection, as well as, resistance transfer among bacteria (Rizzo et al., 2013a). Therefore, the release of ARB into the receiving water should be controlled by an effective disinfection treatment since conventional disinfection processes (e.g., chlorination and UV radiation) are partially effective in controlling ARB spread (Munir et al., 2011; Rizzo et al., 2013b). Consequently, alternative/new disinfection processes should be investigated to reduce the formation of ARB and antibiotic resistance of survived colonies.

Advanced oxidation processes (AOPs) (e.g., Fenton, photo-Fenton, TiO_2 photocatalysis, UV/O_3, UV/H_2O_2 etc.) have been successfully investigated for the removal of a wide range of contaminants (Zapata et al., 2010; Rizzo, 2011; Sannino et al., 2013; Murcia et al., 2013). However, despite of the extensive literature on the inactivation of microorganisms by AOPs (Malato et al., 2009; Dunlop et al., 2011), only a few studies have focused on the effect of AOPs on ARB (Tsai et al., 2010; Öncü et al., 2011) and particularly on indigenous ARB (Rizzo et al., 2014). Among AOPs, TiO_2 photocatalysis has recently emerged as an effective water disinfection option, an alternative to disinfection with chemicals which results in the formation of harmful and toxic disinfection by-products (Richardson et al., 1996; Fernández et al., 2005; Rizzo, 2009a). Despite an increasing number of scientific studies in photocatalysis has demonstrated its efficacy in water and wastewater treatment, industrial applications are still limited possibly because of some technical/economical limitations. Among these, the removal of the catalyst after treatment is a challenge not yet successfully addressed (Rizzo, 2009b).

The TiO_2 photocatalytic process has been found to be effective in the inactivation of a wide range of pathogens in water, including indicator bacteria, e.g. *Escherichia coli* (Bekbölet, 1997), bacterial spores (Dunlop et al., 2008) and protozoa (Sunnotel et al., 2010). However, the effectiveness of TiO_2 photocatalysis on the inactivation of ARB and their resistance in wastewater effluents has not been investigated. *E. coli* are typically detected in UWWTP effluents, and *E. coli* strains resistant to antibiotics have been isolated (Reinthaler et al., 2003; Ferreira da Silva et al., 2007; Rizzo et al., 2012). Therefore, the treatment of UWWTP effluent by new/alternative disinfection processes to effectively control the extent of ARB release in wastewater-polluted stream is in need of investigation.

In the present study, the effect of TiO_2 photocatalysis process on a multi drug resistant *E. coli* strain selected from the effluent of an UWWTP was investigated under different irradiation conditions (using artificial and solar light) and photocatalysts loadings. Antibiotic resistance of *E. coli* strain to ciprofloxacin (CIP), cefuroxime (CEF), tetracycline (TET) and vancomycin (VAN), before and after photocatalytic treatment, was evaluated by Kirby–Bauer method. In contrast to previous studies on water disinfection, in this study we introduce the concept of reactor optical thickness to explain the rates of disinfection observed.

2. Materials and methods

2.1. Wastewater samples

Wastewater samples were collected from a large UWWTP (250,000 equivalent inhabitants) located in southern Italy, from the effluent of the biological treatment process (activated sludge) just upstream of the disinfection unit currently employing chlorination. Samples were collected in sterilized 1 L amber glass bottles. The wastewater was characterized as follows: pH 7.9, BOD_5 10.0 mg L^{-1}, COD 23.3 mg L^{-1}, TSS 32.5 mg L^{-1}, redox potential 63.6 mV, conductivity 1105 $\mu S\ cm^{-1}$.

2.2. Inoculum and sample preparation

Multi drug resistant *E. coli* strain was selected according to the methodology published in Rizzo et al. (2012). Briefly, 50 mL of wastewater sample were filtered through 0.45 μm membrane filters (Millipore, Billerica, MA, USA) and then cultivated (24 h incubation time at 44 °C) in tryptone bile X-glucuronide (TBX) agar medium (Oxoid, Basingstoke, UK), a selective, chromogenic medium for the detection and enumeration of *E. coli*. Ten colonies were randomly collected from TBX agar medium after the incubation period and used in the subsequent step for the selection of the resistant strains. Each colony was cultivated (24 h incubation time at 37 °C) in four different tryptone soya agar (TSA) media (Oxoid, Basingstoke, UK) prepared with a mixture of three antibiotics (amoxicillin, ciprofloxacin, sulphametoxazole). The antibiotics concentrations were chosen according to the respective minimum inhibiting concentrations for *E. coli* listed in "Clinical and Laboratory Standards Institute" documentation (CLSI, 2011). The *E. coli* strains were taken from the Petri dish, transferred in 15% glycerol tryptic soya broth (TSB) (Oxoid, Basingstoke, UK) and frozen at − 20 °C. The selected strains were identified by the Rapid One System method (Remel, Lenexa, KS, USA). In particular, among the selected colonies, the *E. coli* strain that showed the higher resistance, which was growth on the medium enriched with 1 mg L^{-1} of ciprofloxacin, 8 mg L^{-1} of amoxicillin, 32 mg L^{-1} of sulphametoxazole (Rizzo et al., 2013b) was used for the photocatalytic tests.

Wastewater samples were first autoclaved (15 min at 121 °C) to remove indigenous bacteria and then inoculated with the selected *E. coli* strain. The *E. coli* strain was unfrozen and transferred into 10 mL physiological solution to achieve 10^7 CFU 100 mL^{-1} (0.5 McFarland). The physiological solution was then added to 500 mL wastewater sample.

2.3. TiO_2 photocatalysis tests

Photocatalytic experiments were carried out in a 2.2 L cylindrical glass reactor (13.0 cm in diameter) filled in with the