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Research article

Solar photocatalysis as disinfection technique: Inactivation of *Klebsiella pneumoniae* in sewage and investigation of changes in antibiotic resistance profile

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ABSTRACT

The presence of pathogenic microorganisms in wastewater and their resistant nature to antibiotics impose effective disinfection treatment for public health and environmental protection. In this work, photocatalysis with metal-doped titania under artificial and natural sunlight, chlorination and UV-C irradiation were evaluated for their potential to inactivate *Klebsiella pneumoniae* in real wastewater. Their overall effect on antibiotic resistance profile and target antibiotic resistance genes (ARGs) was also investigated. In particular, Mn-, Co- and binary Mn/Co-TiO_2 were tested resulting in bacterial decrease from 4 to 6 Logs upon 90 min of exposure to simulated solar irradiation. The response of catalysts under natural solar light was insufficient, as only a 2 Log reduction was recorded even after 60 min of treatment. The relative activity of the applied methods for *K. pneumoniae* inactivation was decreased in the order: photocatalysis with the binary Co/Mn-TiO_2 under artificial light > chlorination with dose of 5 mg/L of free chlorine > UV-C irradiation, at an initial bacterial concentration of 10^7 CFU/mL. The applied methods showed various effects on antibiotic resistance profile in residual cells. Among the tested antibiotics (ampicillin, cefaclor, sulfamethoxazole and tetracycline), considerable changes in MIC values were recorded for cefaclor and tetracycline. Resistance of surviving cells after treatment remained in high levels, reflecting the abundance of the corresponding target ARGs, namely tetA, tetM, sul1, blaTEM and ampC. The notable presence of target ARGs post disinfection raises concerns and makes wastewater effluent a carrier of antibiotic resistance elements into the aquatic environment.

1. Introduction

The extensive use of antibiotics in clinical medicine and veterinary practice raises many concerns for public health protection. Although the role of those pharmaceuticals is undoubtful, their applications have exceeded any acceptable limits, leading to an uncontrolled dispersion into the environment (Manaia et al., 2016). The fact that antibiotics are partially decomposed during physical and chemical treatments adds to this trend with enormous environmental impacts (Rizzo et al., 2013). Remnants of antibiotics in aquatic bodies ultimately affect the microbial communities and their structural evolution, with the latter exhibiting acquired resistance to one or more classes of antimicrobial substances. The prevalence of antibiotic resistance in bacterial populations has emerged as one of the pre-eminent public health concerns of the 21st century, particularly as it pertains to pathogenic organisms (Andersson and Hughes, 2010). The development of resistance is induced by the respective genes, which propagate and spread very rapidly among microbial communities. Antibiotic resistance genes (ARGs) have been detected in various environments such as surface water, groundwater, sediments, wetlands and sewage, indicating their persistence under extremely different conditions (Mao et al., 2015).

Of particular interest is the acquired resistance in some bacterial species, which are often referred as virulent, opportunistic and

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emerging pathogens, among which *Klebsiella pneumoniae* is prominently included (Makris et al., 2014). It belongs to the family of Enterobacteriaceae and it is a gram-negative, lactose-fermenting bacillus, often reported for its adaptability and resistant nature against bactericidal agents (Baroud et al., 2013; Keynan and Rubinstein, 2007; Sinha et al., 2008). Besides the multi-drug resistance of *K. pneumoniae*, other structural features like the production of extracellular polymers, the potential of lipids alteration in the membrane and considerable cell aggregation, highlight its tolerance during numerous treatment techniques (Bitton, 2011; Burke et al., 2009).

Wastewater treatment plants (WWTPs) offer an ideal setting for the proliferation of antibiotic resistant bacteria (ARB) and dissemination of ARGs, as the applicable conditions allow the growth and development of diverse microorganisms (Aydin et al., 2015). The extent to which treatment and disinfection processes inactivate ARB and eliminate the genes relevant to resistance is still under discussion (Bouki et al., 2013; Jahne et al., 2015). Although conventional methods such as chlorination or UV irradiation prove to be effective in inactivating various microorganisms, there is little information regarding their role regarding the alteration of resistance profile in residual cells after treatment. While complete bacterial inactivation is mostly desired, many bacteria remain post treatment with possible changes/mutations in DNA with various consequences, including the different behavior in the presence of antibiotic compounds (Aydin et al., 2015; Mao et al., 2015; Rizzo et al., 2013; Rodriguez-Mozaz et al., 2015).

Given the persistence of many bacterial species in wastewater like *K. pneumoniae*, it is imperative to explore further the potential of well-established and modern disinfection methods to inactivate them without increasing their antibiotic resistance. Over the last decades heterogeneous photocatalysis has gained attention, as it effectively removes bacteria, parasites and viruses from water and wastewater (Ishiguro et al., 2013; Kubacka et al., 2013; Robertson et al., 2012). Furthermore, optimization of the process involves metal doping of the most widely used catalyst, i.e. TiO_2, which results in the exploitation of visible light (Kubacka et al., 2013; Liou and Chang, 2012; Sethi et al., 2014; Xiao et al., 2015). In contrast, pure titania requires UV irradiation for the excitation and the production of the biocidal reactive oxygen species (ROS) (Ishiguro et al., 2013). Doped-TiO_2, which is visible light-responsive, has shown satisfactory antimicrobial activity towards the inactivation of many bacteria and viruses (McEvoy et al., 2013).

In this perspective, the objectives of the current study were (i) to investigate the potential of cobalt- and manganese-doped titania materials, prepared in previous works (Binas et al., 2012; Venieri et al., 2014), to inactivate *K. pneumoniae* in real wastewater under simulated solar radiation and natural sunlight, (ii) to compare inactivation rates among solar photocatalysis, UV-C irradiation and chlorination, (iii) to study possible changes in bacterial antibiotic resistance profile through treatment and (iv) to detect target ARGs prior and post disinfection.

2. Materials and methods

2.1. Bacterial strain and wastewater samples

The bacterial strain used in the present study was a clinical isolate of *K. pneumoniae*, obtained from fecal material of a hospitalized patient. Biochemical identification of the strain was performed with the API® 20E kit (Biomérieux). The isolated strain was stored in deep fridge (−80 °C) in glucose solution for further disinfection experiments.

Real wastewater samples employed in all disinfection experiments were collected from the municipal wastewater treatment plant (117,500 equivalent inhabitants) located in Chania, W. Crete, Greece. Effluent of the biological treatment process (activated sludge) sludge unit (secondary clarifier) prior to disinfection (chlorination) was sampled from January to August of 2014. The chemical synthesis was as follows: the chemical oxygen demand and dissolved organic carbon were 26 and 7.8 mg/L, respectively, the concentration of chlorides, sulfates, nitrates, nitrites, bicarbonates and total solids were 222.1, 60.3, 25.9, 57.1, 182.1 and 7 mg/L, respectively. The pH had an average value of 7.8. All samples were sterilized in an autoclave before any disinfection treatment and were inoculated with *K. pneumoniae* liquid culture. The desired concentration of bacterial cells in sewage sample in each case was estimated measuring its optical density at 600 nm (Shimadzu UV1240 spectrophotometer). According to McFarland scale, an absorbance of 0.132 corresponds approximately to a cell density of 1.5×10^8 CFU mL^{-1}.

2.2. Metal-doped TiO_2 nanoparticles

Metal-doped nanoparticles used for photocatalytic experiments were synthesized and characterized in previous works (Binas et al., 2012; Venieri et al., 2014). In the present study Mn-, Co- and Mn/Co binary-doped TiO_2 catalysts were used, which were prepared by a co-precipitation method with molar ratio in different concentrations. Specifically, experimental runs were performed with 0.1 wt% Mn:TiO_2, 0.1 wt% Co:TiO_2 and 0.04 wt% Mn/Co:TiO_2. The commercially available titanium dioxide powder (TiO_2 P25), which was used as benchmark was purchased from Degussa - Evonik Corp. with physicochemical characteristics anatase:rutile 75:25, primary particle size of 21 nm and BET area of 50 m^2/g (CAS No: 13463-67-7).

2.3. Disinfection experiments

The first set of disinfection experiments included photocatalytic treatment in a batch type, laboratory scale photoreactor with a solar radiation simulator system (Newport, model 96000) and an irradiated surface of 330 cm^2. The system was equipped with a 150 W xenon ozone-free lamp and an Air Mass 1.5 Global Filter (Newport, model 81094), simulating solar radiation reaching the surface of the earth at a zenith angle of 48.2°. The spectral output of the lamp was from 200 nm to approximately 2400 nm and the simulated radiation contained about 5% UV-A and 0.1% UV-B, according to the data provided by the manufacturer. Radiations with wavelengths lower than 280 nm were cut by the filter. The incident radiation intensity on the photochemical reactor in the UV region of the electromagnetic spectrum was measured 5.8×10^{-7} E/(L.s), which corresponds to an irradiance of 1.31×10^{-2} W/m^2 (Galbavy et al., 2010; Willett and Hites, 2000). In each experimental run 300 mL of wastewater, which were inoculated with *K. pneumoniae* cells, were loaded in the reaction vessel (open, double-walled, cylindrical glass vessel) with the appropriate amount of catalyst. Prior to treatment the bacterial liquid culture was grown overnight at 37 °C in nutrient broth (LABM - code: LAB068), in order to obtain the desired initial bacterial population. The prepared solution was left in the dark under stirring for 30 min for equilibration followed by exposition to solar irradiation. The temperature was maintained at 25±2 °C throughout treatment with a temperature control unit and the external reaction vessel was covered with aluminum foil to reflect irradiation exerting the outer wall of the reaction vessel.

The second set of disinfection experiments was photocatalytic inactivation carried out under natural sunlight. Those runs were conducted within the period of June–July 2014 at the Technical University of Crete campus located at 38°31′ N and 24°04′ E, in clear sunny days with solar irradiance and temperature in the range of