Solar treatment ($\text{H}_2\text{O}_2$, TiO$_2$-P25 and GO-TiO$_2$ photocatalysis, photo-Fenton) of organic micropollutants, human pathogen indicators, antibiotic resistant bacteria and related genes in urban wastewater


PII: S0043-1354(18)30085-X
DOI: 10.1016/j.watres.2018.01.064
Reference: WR 13543

To appear in: Water Research

Received Date: 1 October 2017
Revised Date: 23 December 2017
Accepted Date: 26 January 2018


This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
Solar treatment (H$_2$O$_2$, TiO$_2$-P25 and GO-TiO$_2$ photocatalysis, photo-Fenton) of organic micropollutants, human pathogen indicators, antibiotic resistant bacteria and related genes in urban wastewater

Nuno F.F. Moreira$^{1,2}$, Carlos Narciso-da-Rocha$^3$, M. Inmaculada Polo-López$^4$, Luisa M. Pastrana-Martínez$^1$, Joaquim L. Faria$^1$, Célia M. Manaia$^3$, Pilar Fernández-Ibáñez$^{4,5,*}$, Olga C. Nunes$^2$, Adrián M.T. Silva$^{1,*}$

$^1$Laboratory of Separation and Reaction Engineering - Laboratory of Catalysis and Materials (LSRE-LCM), Faculdade de Engenharia, Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

$^2$LEPABE – Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculdade de Engenharia, Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

$^3$Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Arquiteto Lobão Vital, Apartado 2511, 4202-401 Porto, Portugal

$^4$Plataforma Solar de Almeria – CIEMAT, P.O. Box 22, 04200 Tabernas, Almeria, Spain

$^5$Nanotechnology and Integrated BioEngineering Centre, School of Engineering, University of Ulster, Newtownabbey, Northern Ireland, BT37 0QB, United Kingdom

*Corresponding authors e-mail addresses:

adrian@fe.up.pt (A.M.T. Silva); p.fernandez@ulster.ac.uk (P. Fernández-Ibáñez).

Abstract

Solar-driven advanced oxidation processes were studied in a pilot-scale photoreactor, as tertiary treatments of effluents from an urban wastewater treatment plant. Solar-H$_2$O$_2$, heterogeneous
photocatalysis (with and/or without the addition of H₂O₂ and employing three different photocatalysts) and the photo-Fenton process were investigated. Chemical (sulfamethoxazole, carbamazepine, and diclofenac) and biological contaminants (faecal contamination indicators, their antibiotic resistant counterparts, 16S rRNA and antibiotic resistance genes), as well as the whole bacterial community, were characterized.

Heterogeneous photocatalysis using TiO₂-P25 and assisted with H₂O₂ (P25/H₂O₂) was the most efficient process on the degradation of the chemical organic micropollutants, attaining levels below the limits of quantification in less than 4 hours of treatment (corresponding to Q_{UV} < 40 kJ L⁻¹). This performance was followed by the same process without H₂O₂, using TiO₂-P25 or a composite material based on graphene oxide and TiO₂.

Regarding the biological indicators, total faecal coliforms and enterococci and their antibiotic resistant (tetracycline and ciprofloxacin) counterparts were reduced to values close, or beneath, the detection limit (1 CFU 100 mL⁻¹) for all treatments employing H₂O₂, even upon storage of the treated wastewater for 3-days. Moreover, P25/H₂O₂ and solar-H₂O₂ were the most efficient processes in the reduction of the abundance (gene copy number per volume of wastewater) of the analysed genes. However, this reduction was transient for 16S rRNA, _intI1_ and _sulI_ genes, since after 3-days storage of the treated wastewater their abundance increased to values close to pre-treatment levels. Similar behaviour was observed for the genes _qnrS_ (using TiO₂-P25), _bla_{CTX-M}_ and _bla_{TEM}_ (using TiO₂-P25 and TiO₂-P25/H₂O₂). Interestingly, higher proportions of sequence reads affiliated to the phylum _Proteobacteria_ (Beta- and Gammaproteobacteria) were found after 3-days storage of treated wastewater than before its treatment. Members of the genera _Pseudomonas, Rheinheimera_ and _Methylotenera_ were among those with overgrowth.

**Keywords:** Solar advanced oxidation processes; urban wastewater; faecal indicators; antibiotic resistance genes; bacterial community composition.
1. Introduction

Conventional urban wastewater treatment plants (UWWTPs) are not specifically designed for the removal of organic micropollutants, and in many cases neither for effective disinfection (removals of bacterial loads up to 2 log cycles) which considers the inactivation of bacteria that can contribute to the spread of antibiotic resistant bacteria (ARB) and antibiotic resistant genes (ARG) into the environment. These contaminants can reach the natural waters, such as surface and ground waters that are serving as drinking water sources (Fatta-Kassinos et al. 2011, Manaia et al. 2016). Moreover, the continuous disposal of antibiotics and related products into the environment can lead to the development and proliferation of ARB, decreasing the efficiency of these antibiotics when supplied to human and animals (Ferro et al. 2016, Rizzo et al. 2013). Since high bacterial density can be found in effluents of UWWTPs (i.e., bacterial cells are close to each other), horizontal gene transfer and selection of ARB can be considered important mechanisms for ARG enrichment (Davison 1999). The dissemination of these contaminants urges the development of new technologies able to improve the simultaneous removal of organic micropollutants and microorganisms of concern.

Advanced oxidation processes (AOPs) are conceptually based on the generation of the highly reactive hydroxyl radicals (HO\(^\cdot\)), but other reactive species can also be formed. AOPs, such as UVC/H\(_2\)O\(_2\) and ozonation, have been widely studied for the removal of hazardous organic chemical compounds and disinfection, and they have been applied as viable solutions to enhance the quality of secondary effluents before discharged or reused (Ribeiro et al. 2015). However, several drawbacks have been associated to the aforementioned processes. For instance, ozonation-based treatments are known to produce bromate (from bromide), which is classified as a “possible human” carcinogen (Xiao et al. 2017), whereas the processes dependent on high/medium vapour pressure lamps (such as UV\(_{254\,nm}\)) have higher operating costs and they are less environmentally friendly than those implementing solar radiation. Furthermore, in a recent study (Becerra-Castro et al. 2016), we have shown that oxidation processes (such as UV\(_{254\,nm}\),...
ozonation and photocatalytic ozonation) might have the potential to act selectively over some bacterial groups. In that bench-scale study, irrespective of the type of treatment used, it was observed a significant modification of the residual bacterial community after 3-days storage, which was characterized by higher proportions of *Proteobacteria* (*Gamma-* and *Betaproteobacteria*) than those observed in non-treated wastewater. This is an example of bacterial community disturbance induced by a disinfection treatment, which eventually may affect negatively the biological quality of the final stream if the reduction of the bacterial diversity and disequilibrium of the microbial ecosystem favour the possible selection of ubiquitous bacteria associated with acquirement and spread of virulence or ARGs (Becerra-Castro et al. 2016).

Among the different AOPs, those driven by natural sunlight have raised a great interest during last decades (i.e. natural solar radiation as source of photons), reducing the need to costly lamps or maintenance of systems. Photocatalysis with irradiated titanium dioxide (*TiO*$_2$) and the photo-Fenton process have demonstrated to be powerful wastewater treatment options. A number of experimental studies are available in literature recognizing the high potential of these solar techniques for water decontamination and disinfection of a wide range of water sources (Malato et al. 2009, Moreira et al. 2016, Pablos et al. 2013, Polo-López et al. 2014, Sousa et al. 2017, Yang et al. 2014). Especially interest for water disinfection has attracted the solar photo-assisted treatment with *H*$_2$*O*$_2$, which has achieved fast inactivation of several types of microorganisms in water (García-Fernández et al. 2012, Polo-Lopez et al. 2011, Sichel et al. 2009). In addition, the development of solar Compound Parabolic Collector (CPC) reactors has resulted in the development of technological solutions with increased efficiency for water treatment (Giannakis et al. 2016, Malato et al. 2009). However, the performance of these solar-based processes has not been investigated in terms of changes of the bacterial community composition and simultaneous removal of organic contaminants and ARB&ARG.
Thus, the present study aims at comparing the efficiency of different solar-driven AOPs on the simultaneous removal of micropollutants and disinfection of a secondary effluent of an UWWTP. Solar-H$_2$O$_2$, heterogeneous photocatalysis (with and/or without the addition of H$_2$O$_2$) and the photo-Fenton process were tested using a pilot-plant CPC solar photoreactor. For the first time, the performance of each process was assessed based on the removal efficiency of organic contaminants and ARB&ARG, as well as on the changes of the bacterial community composition. Microbial characterization was performed before treatment, after 5 hours of treatment and after 3-days storage of treated wastewater at room temperature. Thus, the simultaneous removal of chemical and biological contaminants by using solar-driven AOPs at pilot-scale and considering possible changes in the bacterial community, which can affect the water quality, is the main novelty of the present work.

2. Materials and methods

2.1 Chemicals and materials

Degussa (Aeroxide) TiO$_2$-P25 catalyst from Evonik Corporation (hereafter referred to as P25) and a composite consisting of TiO$_2$ and 4.0 wt.% of graphene oxide (GO-TiO$_2$) – respective preparation method and characterization described elsewhere (Pastrana-Martínez et al. 2012) – were used to conduct heterogeneous photocatalytic experiments. For comparative purposes, bare TiO$_2$ was also prepared following the same method used for GO-TiO$_2$, but without the addition of GO (hereafter referred to as TiO$_2$). The H$_2$O$_2$ 30% (w/v) solution, analytical grade sulphuric acid (H$_2$SO$_4$, 98%), bovine liver catalase and ferrous sulphate heptahydrate (FeSO$_4$·7H$_2$O) were obtained from Riedel-de Haën (Germany), Merk (Germany), Sigma-Aldrich (USA) and PANREAC (Spain), respectively. Carbamazepine (CBZ), sulfamethoxazole (SMX) and diclofenac (DFC) were all high-purity grade (>99%), and purchased from Sigma-Aldrich (Spain). Stock solutions were prepared by dissolving the individual compounds (2.5 g L$^{-1}$) in methanol due to water solubility limitations. Required volumes of the stock solutions were directly added
to the urban wastewater samples into the CPC pilot reactor to obtain the initial concentration of 100 µg L\(^{-1}\) of each organic micropollutant. Methanol (J.T.Baker) and acetonitrile (Sigma-Aldrich) were HPLC-grade. Ultrapure water was supplied by a Milli-Q water system.

2.2 Municipal wastewater treatment plant samples

All solar-driven treatments were carried out using urban wastewater samples collected (every day in batches of 60 L) after the secondary treatment from the UWWTP of El Bobar (Almería, Spain), and stored at 4 °C no more than 2 hours before solar experiments. The UWWTP uses conventional activated sludge plus decantation as secondary treatment. In 2015 (year of the sampling campaign), it produced an average of secondary effluent daily flow of ca. 33,000 m\(^3\), with a capacity of 100,000 inhabitant-equivalent. The main physicochemical characteristics of the effluent, including turbidity, pH, conductivity, dissolved inorganic carbon (DIC), dissolved organic carbon (DOC) and inorganic ions concentration, are listed in Table S1 (Supplementary Information).

2.3 Pilot scale CPC photoreactor and solar experiments

All the solar-driven oxidation processes were performed in a pilot-scale CPC photoreactor, at Plataforma Solar de Almería (PSA), Spain (37°84’N and 2°34’W), on sunny days between June and August 2015, with a duration of 5 hours. The configuration of the CPC photoreactor is described elsewhere (Rodríguez-Chueca et al. 2014). The CPC photoreactor tube module, tilted at an angle of 37° relative to the horizontal plane, is connected to a recirculation tank and a centrifugal pump. The water flow rate was set at 10 L min\(^{-1}\). The total volume of the photoreactor was 20 L, while the illuminated volume and the irradiated collector surface area were 15 L and 1 m\(^2\), respectively. Target chemical and biological contaminants were simultaneously monitored in the same experiment.
In heterogeneous photocatalysis (P25, TiO$_2$ and GO-TiO$_2$) a catalyst load of 200 mg L$^{-1}$ was used. In photo-Fenton (Fe$^{2+}$/H$_2$O$_2$), ferrous sulphate heptahydrate was used as source of 10 mg L$^{-1}$ of Fe$^{2+}$. In H$_2$O$_2$ assisted processes (H$_2$O$_2$, Fe$^{2+}$/H$_2$O$_2$, P25/H$_2$O$_2$ and GO-TiO$_2$/H$_2$O$_2$), the initial concentration of H$_2$O$_2$ was 20 mg L$^{-1}$ reached by adding the H$_2$O$_2$ 30% (w/v) solution. Different amounts of H$_2$O$_2$ added to the system may have different effect on the removal efficiencies (Cai and Hu 2017, Polo-López et al. 2014). The catalyst and H$_2$O$_2$ concentrations were selected considering the optimization done in our previous studies with solar-driven oxidation processes (Fernández-Ibáñez et al. 1999, Polo-López et al. 2014). Immediately after the collection of a sample, residual H$_2$O$_2$ was eliminated by adding a freshly prepared solution of bovine liver catalase (0.1 g L$^{-1}$) in a ratio 0.1/5.0 (v/v). Photolysis (Blank) assays were performed to study the effect of solar radiation without the addition of any catalyst or reactant.

Besides the experimental time, the accumulated solar UVA energy received in the solar reactor per unit of treated water volume ($Q_{UV}$, kJ L$^{-1}$) was considered for comparison of the treatment efficiencies (Malato et al. 2009) and calculated using Eq. (1):

$$Q_{UV,n} = Q_{UV,n-1} + \frac{\Delta t \cdot UV_{G,n} \cdot A_r}{V_t}; \Delta t_n = t_n - t_{n-1}$$  \hspace{1cm} (1)

where $UV_{G,n}$ is the global UV irradiance (W m$^{-2}$) averaged during exposure time; $t_n$ the exposure time (s); $V_t$ the total reactor volume (L); and $A_r$ the illuminated reactor surface (m$^2$).

A global UVA (300–400 nm, Model CUV4, Kipp & Zonen, Netherlands) pyranometer tilted 37° was used to measure the solar radiant UVA rate incident (W m$^{-2}$) as described elsewhere (Rodríguez-Chueca et al. 2014). The average value of solar UVA irradiance was 40 W m$^{-2}$.

Before treatment, the CPC photoreactor was covered by an opaque sheet while the wastewater and reagents were added to the reactor and recirculated during 15 min to guarantee homogenisation. Samples were collected each hour over the treatment. During this period, temperature (Checktemp, Hanna instruments, Spain) and pH (multi 720, WTW, Germany) were measured. The temperature of the wastewater varied from 16.6 ± 1.5 °C to 43.2 ± 4.2 °C.
2.4 Chemical analysis

High performance liquid chromatography (HPLC) was used to analyze the evolution of the target organic micropollutants using an apparatus from Agilent Technologies (series 1260, Palo Alto, CA, USA), equipped with a diode array detector (UV-DAD) and a C-18 column. The mobile phase consisted of 90% formic acid aqueous solution at 25 mM and 10% acetonitrile. A linear gradient was used from 10% to 85% of acetonitrile during 13 min at a flow rate of 1 mL min\(^{-1}\). The injection volume was set at 100 µL. Before injection, samples were filtered through a 0.2 µm syringe-driven filter, and afterwards the filter was washed with 1 mL of acetonitrile to remove the adsorbed compounds. CBZ, SMX and DFC were selected as model organic micropollutants since they have been frequently found in aquatic environments, including wastewater, surface and groundwater and even in drinking water (Barbosa et al. 2016). The detection wavelength values were 267 nm for both CBZ and SMX, and 273 nm for DFC. The limits of quantification (LOQ) were 4.7, 6.2 and 4.1 µg L\(^{-1}\) for CBZ, SMX and DFC, respectively.

DX-600 and DX-120 (Dionex Corporation, Sunnyvale, CA) equipments were used for quantification of anions and cations, respectively. DOC and DIC measurements were performed using a 5050 A TOC (Shimadzu Corporation, Kyoto, Japan) analyzer, after sample filtration using 0.2 µm syringe filters. Turbidity was determined with a turbidimeter (Model 2100N, Hach, USA). The \(\text{H}_2\text{O}_2\) concentration was determined by a spectrometric method, as described elsewhere (Polo-López et al. 2011), and the \(\text{Fe}^{2+}\) concentration by the ISO 6332 method. Natural iron was not detected in any of the urban wastewater samples by using this method, i.e. a spectrophotometric technique with phenanthroline/acetic acid (UV–Vis measurements, limit of detection 0.05 mg L\(^{-1}\)).

2.5 Microbiological cultivation, DNA extraction, qPCR and bacterial community analysis

To assess the disinfection efficiency, cultivable faecal indicator bacteria, targeted ARB and selected ARGs were quantified before and immediately after the 5 hours-treatment, and after 3-
days of storage of treated wastewater at room temperature. The abundance of faecal coliforms, enterococci and their tetracycline and ciprofloxacin resistant counterparts was assessed by using the membrane filtration method. Faecal coliforms and enterococci are used worldwide as indicators of faecal contamination, in particular to assess the microbiological water quality (APHA 2005, ISO9308-1 2000). In addition, the ubiquity of these bacteria in human impacted environments and their high persistence in the environment, as well as genome plasticity, make of faecal coliforms and enterococci important tracers to assess the antibiotic resistance status of environmental samples. Furthermore, in these groups high resistance prevalence has been observed for antibiotics such as tetracycline and ciprofloxacin in aquatic environments, including wastewater (Vaz-Moreira et al. 2014). After serial 10-fold dilutions in sterile saline solution (0.85% NaCl), 100 mL of each dilution was filtered through cellulose membrane filters (0.22 µm porosity; Whatman, UK). The filtering membranes were incubated on selective media for each target bacterial groups - membrane Faecal Coliforms agar (m-FC) (Difco, 30 °C, 24 hours) for faecal coliforms, and Slanetz & Bartley agar (S&B) (Difco, 30 °C, 48 hours) for enterococci. In addition, the prevalence of ARB was assessed in the same media supplemented with tetracycline (Fluka, 16 mg L\(^{-1}\)) and ciprofloxacin (Applichem, 1 mg L\(^{-1}\)). The antibiotic stock solutions were sterilized by filtration (0.2 µm syringe driven-filters).

The use of adequate indicators of antibiotic resistance in both culturable and non-culturable bacteria has been recommended (Berendonk et al. 2015). For culture-independent assays, a volume of 250 mL of each sample (before and after the 5 hours treatment, and after 3-days of storage) was filtered through polycarbonate membranes (0.22 µm porosity; Whatman, UK). DNA was extracted using the commercial kit PowerWater® DNA Isolation (MO BIO Laboratories, Inc., USA) and stored at -20 °C. These extracts were used for the quantification of the house keeping gene 16S rRNA, \textit{intII} (related with ARG mobilization) and a set of ARGs encoding resistance to different classes of antibiotics frequently detected in wastewater (Vikesland et al. 2017) by quantitative PCR (qPCR) of samples collected before treatment, immediately after
treatment, and after 3-days of storage. In addition, the same extracts were also used for the bacterial community analysis, in this case except for the samples collected immediately after treatment, due to DNA scarcity. The qPCR (StepOne™ Real-Time PCR System; Life Technologies, Carlsbad, CA) assays were performed according to the conditions shown in Table S2 - Supplementary Information (Bibbal et al. 2007, Denman and McSweeney 2006, Goldstein et al. 2001, Marti and Balcázar 2013, Marti et al. 2014, Pei et al. 2006, Volkmann et al. 2004), and as described elsewhere (Narciso-da-Rocha et al. 2014).

The bacterial community composition was analysed based on the hypervariable V3/V4 region (forward primer Bakt_341F 5′-CCTACGGGNGGCWGCAG-3′ and reverse Bakt_805R 5′-GACTACHVGGGTATCTAATCC-3′) of 16S rRNA gene Illumina sequencing (Genoinseq, Cantanhede, Portugal). Nucleotide sequencing data were processed and analysed using the QIIME pipeline (Caporaso et al. 2010). Briefly, sequences shorter than 250 bp and with average quality scores lower than 25 were eliminated, and bases with average quality lower than 25 in a window of 5 bases, were trimmed using the software PRINSEQ (Schmieder and Edwards 2011). Chimeric sequences were identified and removed using USEARCH v6.1 (Edgar 2010). Freechimeric sequences were further grouped into operational taxonomic units (OTUs) using USEARCH v6.1 (Edgar 2010) with a phylotype threshold of ≥ 97% sequence similarity and were taxonomically assigned using QIIME default values. The sequences comprising each OTU were aligned using PyNAST (Caporaso et al. 2010) and were taxonomically classified using Greengenes Database version 13_8 (updated: August 2013) (DeSantis et al. 2006). As a variable number of sequences was obtained between samples, the alpha diversity indices Shannon, phylogenetic diversity (PD) whole tree, and Simpson were calculated after rarefying to 54,771 sequences per sample (value of the smallest dataset) (Faith 1992, Shannon and Weaver 1963, Simpson 1949). An alpha diversity index represents the diversity within a particular community and it is affected by its size, in particular by the number of different species and the abundance of each one (Whittaker 1972). The cumulative sum scaling (CSS) normalization procedure was
applied to the sequence data to assess the beta diversity patterns (Paulson et al. 2013). The weighted UniFrac metric distances (Lozupone and Knight 2005) were calculated in the QIIME pipeline and the results shown as Principal Coordinates Analysis (PCoA) biplots that include the position of the ten most prevalent bacterial classes. Correlations between the relative abundance of populations at different taxonomic levels were analysed using the statistics software STAMP v2.1.3 (Parks et al. 2014).
3. Results and discussion

3.1 Degradation of organic micropollutants

The evolution of the concentrations of targeted organic micropollutants (i.e., CBZ, SMX and DFC) spiked in urban wastewater under solar-driven oxidation treatments are shown in Fig. 1. Control experiments were performed under the same conditions but without the addition of any catalyst or H$_2$O$_2$ (i.e., Blank = photolysis). CBZ and SMX were very resistant upon irradiation in the absence of a catalyst or H$_2$O$_2$ (only 20 ± 6% and 17 ± 4% of removal, respectively). In contrast, DFC was efficiently removed by photolysis after 4 hours. This result was expected since the DFC absorption spectrum (not shown) has a tail entering well above the 300 nm (Moreira et al. 2015).

The heterogeneous photocatalysts (P25, TiO$_2$ and GO-TiO$_2$), without the addition of H$_2$O$_2$, converted the targeted organic micropollutants in the following order of decreasing efficiency: P25 > GO-TiO$_2$ > TiO$_2$ (Fig. 1). The high photocatalytic efficiency of GO-TiO$_2$ on the degradation of different types of organic micropollutants in synthetic matrices has been already demonstrated in our previous studies, namely for: diuron, alachlor, isoproturon and atrazine – pesticides classified by the EU as priority pollutants (Cruz et al. 2017); microcystin-LA – cyanotoxin (Sampaio et al. 2015); bisphenol A – xenoestrogen (Maroga Mboula et al. 2013); diphenhydramine – antihistamine pharmaceutical, and methyl orange – azo dye (Pastrana-Martínez et al. 2012). The GO-TiO$_2$ photocatalyst has been much more active than P25 under Vis-light illumination, but under near UV-Vis radiation the activity of this type of photocatalysts depends on the target pollutant. The better performance of GO-TiO$_2$ in comparison to bare TiO$_2$ has been attributed to the good assembly and interfacial coupling between TiO$_2$ and GO sheets as well as the respective quenching of photoluminescence (inhibiting charge recombination) (Pastrana-Martínez et al. 2012). In the present study, P25 was the most efficient photocatalyst for the degradation of the organic micropollutants, most probably, because of the better performance of P25 under UV radiation.
The addition of H$_2$O$_2$ to the P25 photocatalytic system, increased the degradation efficiency of the targeted micropollutants. H$_2$O$_2$ captures and reacts with the photoinduced surface electrons (suppressing the electron/hole recombination) and it also reacts with the superoxide radical anions, both pathways leading to the formation of additional hydroxyl radicals (Kositzi et al. 2004, Pablos et al. 2013). There is markedly a competing process, which may be mediated by the active surface of the photocatalyst. Interestingly, the removal efficiency decreased when H$_2$O$_2$ was added to the photocatalytic system containing GO-TiO$_2$. Degradation of GO-TiO$_2$ may eventually occur, for instance by the H$_2$O$_2$ attack to the underlying C-C bonds in the superficial defect sites of GO (Xing et al. 2014).

Solar-H$_2$O$_2$ and photo-Fenton processes (Fe$^{2+}$/H$_2$O$_2$) also led to very modest removals of CBZ and SMX (Figs. 1a and b, respectively). One of the downsides of photo-Fenton is the formation of iron sludge due to the precipitation of iron hydroxide at neutral pH. In this work, the pH was adjusted to a circumneutral value (5.5) by using H$_2$SO$_4$, which could explain the low photo-Fenton efficiency that is known to be maximized at pH values around 3 (Agulló-Barceló et al. 2013, García-Fernández et al. 2012, Giannakis et al. 2017). In a previous work (Klamerth et al. 2010), solar photo-Fenton (also operated at mild pH conditions) was effective for the degradation of organic micropollutants, but the total inorganic carbon was removed by sample acidification since it competes with the organic contaminants for hydroxyl radicals (Klamerth et al. 2009).

Overall, P25/H$_2$O$_2$ followed by the P25 and GO-TiO$_2$ photocatalytic processes were the best performing treatments for removal of the targeted organic micropollutants. Regarding the mineralization of the organic matter present in the urban wastewater, P25/H$_2$O$_2$, solar-H$_2$O$_2$, and the photo-Fenton process were the most efficient treatments (DOC removals always around 23 ± 3%), other processes removing no more than ca. 10% of the initial DOC. However, due to the chemical complexity of this matrix, and the differences between the DOC (in the range of mg L$^{-1}$)
and the concentration of the studied organic pollutants (in the range of \( \mu g \) L\(^{-1} \)), it is not possible to correlate the DOC and micropollutants removals.

### 3.2 Bacteria inactivation and reactivation

The performance of different solar-driven processes was assessed before and over the treatment, in terms of removal of total faecal coliforms (Fig. 2a), enterococci (Fig. 2c) and respective fraction of resistant populations (Figs. 2b and d). The reduction of the bacterial indicators loads was observed in all the treatments, with the highest inactivation rates leading to values below or close the LOD (1 CFU 100 mL\(^{-1} \)), for the processes where \( H_2O_2 \) was used (\( H_2O_2, Fe^{2+}/H_2O_2, P25/H_2O_2, GO-TiO_2/ H_2O_2 \)). Among these, and despite the iron precipitation, the photo-Fenton process was the most efficient treatment on the reduction of resistant and non-resistant faecal coliforms and enterococci (Figs. 2a-d, \( Fe^{2+}/H_2O_2 \)). However, photo-Fenton showed similar disinfection profiles to solar-\( H_2O_2 \) for faecal coliforms (Figs. 2a and b) and to P25/\( H_2O_2 \) for enterococci (Figs. 2c and d). These three processes removed those bacteria for \( Q_{UV} < 30 \) kJ L\(^{-1} \), suggesting that \( H_2O_2 \) plays a major role on disinfection, including in the case of solar-\( H_2O_2 \) and photo-Fenton that were not effective for the removal of all the organic micropollutants. In contrast, moderate inactivation rates were observed for photolysis (Figs. 2a, b and c, Blank), except for antibiotic resistant enterococci that were reduced to values below the LOD (Fig. 2d, Blank). Interestingly, P25/\( H_2O_2 \) showed high efficiency on the removal of organic micropollutants and resistant and non-resistant enterococci, whereas the efficiency for inactivation of resistant and non-resistant faecal coliforms was not so high in comparison with solar-\( H_2O_2 \).

**FIGURE 2**

The accepted hypotheses and mechanism that explain the inactivation of microorganisms by exposure to solar-\( H_2O_2 \) is based on the accumulated damages inside cells by internal cellular injures occurring under sunlight and accelerated in the presence of \( H_2O_2 \). It is well accepted that
solar radiation produces internal damages affecting different intracellular vital components leading to bacterial death or lack of viability (Aguas et al. 2017). A recent study attributed bacterial inactivation during solar photolysis to the combined effect of intracellular production of reactive oxygen species by UV photons absorption and water temperature increase (Castro-Alférez et al. 2017). When H$_2$O$_2$ is added, it may diffuse inside bacteria cells promoting additional internal photo-reactions with naturally present iron and other metals via Fenton and Fenton–like reactions, activating, thus, a photo-Fenton cycle under sunlight at intracellular level also (Aguas et al., 2017). Both photo-effects act jointly producing an accelerated disinfection that has been also reported to be very efficient for other types of bacteria, viral indicators, and fungi including, *Escherichia coli* and *Enterococcus faecalis* (Rodríguez-Chueca et al. 2014), *Legionella jordanis* (Aguas et al. 2017), F-specific RNA bacteriophage (Agulló-Barceló et al. 2013), *Fusarium* sp. (Polo-López et al. 2014, Sichel et al. 2009), *Phytophthora capsici* (Polo-López et al. 2013), *Curvularia* sp. (Aguas et al. 2017), and several antimicrobial resistant bacteria (Fiorentino et al. 2015).

Although H$_2$O$_2$ assisted processes performed better that non-assisted ones, heterogeneous photocatalysis without H$_2$O$_2$ also performed quite well on the inactivation of total or resistant populations of faecal coliforms and enterococci. Among these processes, the GO-TiO$_2$ composite was the most efficient catalyst for the removal of total and resistant populations of faecal coliforms (Figs. 2a and b, GO-TiO$_2$). The good efficiency of photocatalytic disinfection using this type of composites (but prepared by other methods) and under visible radiation only was already shown in previous studies (Cruz-Ortiz et al. 2017, Fiorentino et al. 2015). Its high photocatalytic activity has been attributed to the improvement in charge separation since GO may promote the electron transfer with TiO$_2$ particles, acting as an electron bridge, and to the decrease of the bandgap energy of the composite catalysts as well as to an enhancement of the adsorptive properties. These authors concluded that hydrogen peroxide, hydroxyl radicals, and singlet oxygen were the main species involved in the disinfection process under UV–Vis irradiation and
only singlet oxygen under visible illumination (Cruz-Ortiz et al. 2017). Moreover, very recently (Karaolia et al. 2018), the photocatalytic efficiency of P25 (in the absence of H$_2$O$_2$) was compared to that of a TiO$_2$-reduced GO composite on the removal of three organic micropollutants (including SMX) and E. coli resistant and non-resistant to antibiotics in urban wastewater. P25 performed better than TiO$_2$-reduced GO for the photocatalytic degradation of SMX, but higher bacteria permanent inactivation was observed in the presence of the composite, in accordance with our results. However, in the same study, TiO$_2$-reduced GO performed better than P25 for the removal of erythromycin (i.e. the opposite of that observed for SMX), the photocatalytic efficiency thus depending on the target organic molecule. In our particular case, P25 was better than the GO-TiO$_2$ composite for the removal of all the studied organic micropollutants (CBZ, SMX and DFC).

Since microbial inactivation, monitored via culture-based methods, can be transient (Moreira et al. 2016, Spuhler et al. 2010, Zhao et al. 2014), further assays testing the regrowth capacity after 3-days storage of the treated wastewater at room temperature were performed. Bacterial reactivation is influenced by factors such as the storage conditions, temperature, availability of nutrients and the UV dose, among others (Giannakis et al. 2014, Ubomba-Jaswa et al. 2009). The bacterial loads before, after the treatment and after 3-days storage at room temperature are shown in Figs. 3a and b for faecal coliforms and Figs. 3c and d for enterococci. No regrowth was observed in stored wastewater treated by the H$_2$O$_2$-assisted processes, and total faecal coliforms and enterococci as well as their ARB counterparts were kept below the detection limit. In stored wastewater treated by heterogeneous photocatalysis without H$_2$O$_2$ (P25, TiO$_2$ and GO-TiO$_2$), the bacterial loads of these groups were 2 to 3 log values lower than before the treatment. Similar observations were registered for the control (Blank = photolysis). These results indicate the inability of these bacterial groups to recover to values close to those found in raw wastewater after the tested solar-driven processes. This can be also explained by the mode of action attributed to solar-H$_2$O$_2$ disinfection, where oxidative damages alter the bacteria viability at intracellular
level, as proven in the literature (Aguas et al. 2017). In such report, an EMA-qPCR method for
the detection of membrane integrity damages of *L. jordanis* cells under two photo-oxidative
processes was used, i.e. solar-H$_2$O$_2$ and P25 with solar radiation. It was confirmed the well-
accepted mechanism of heterogeneous (P25) photocatalysis via oxidative attacks of the external
cell membrane, whereas the mechanism for solar-H$_2$O$_2$ was based on internal photochemical
reactions. This may explain the results reported in Fig. 3 on the non-recover capability of ARB
when H$_2$O$_2$ was used in these treatment processes, while they can regrow when H$_2$O$_2$ is not in the
media (Aguas et al. 2017). In general, the selected solar-driven oxidation processes were effective
in reducing faecal indicators, including those resistant to tetracycline and ciprofloxacin, from
wastewater samples.

**FIGURE 3**

3.3 Effect of disinfection on ARGs and bacterial community composition

Considering that the bacterial community is much more diverse and complex than that assessed
based on the cultivation methods used, and that some bacteria may be injured and hence, unable
to grow, culture-independent methods were carried out to give additional insights on the
disinfection effectiveness of the studied solar-driven processes. Since the main purpose of this
work was the simultaneous removal of chemical and biological contaminants, the processes
showing better performance on the degradation of organic micropollutants (i.e., P25/H$_2$O$_2$, P25
and GO-TiO$_2$ photocatalytic processes), disinfection (P25/H$_2$O$_2$ and solar-H$_2$O$_2$), and also the
reference process (photolysis), were selected for further investigation based on culture-
independent methods - Fe$^{2+}$/H$_2$O$_2$ was not chosen due to its bad performance for degradation of
the organic micropollutants (Fig. 1).

Among the analysed genes (i.e., 16S rRNA, *intI1*, qnrS, *bla$_{CTX-M}$*, *sul1*, *bla$_{TEM}$* and *vanA*), only
*vanA* was below the LOD before treatment (not shown). For the other genes, P25/H$_2$O$_2$
photocatalysis and solar-H$_2$O$_2$ were the most efficient processes (i.e., lower abundance after
treatment), both leading to log average reduction of 1 value, except for *bla$_{CTX-M}$* (3 log reduction)
However, after 3-days storage, regardless the treatment used, the abundance of 16S rRNA gene, a house keeping gene of prokaryotes, was close or even higher (up to 1 log for P25/H$_2$O$_2$) than those found before treatment, suggesting the ability of some bacteria to recover after the treatment. Similar results were observed for intII and sulI genes, encoding integrase and conferring resistance to sulphonamides, respectively. Among the studied processes, only solar-H$_2$O$_2$ and GO-TiO$_2$ prevented the reactivation of bla$_{CTX,M}$ and bla$_{TEM}$ genes (encoding resistance to beta-lactams) above the pre-treatment levels. For the qnrS gene (a plasmid-mediated quinolone resistance gene), besides these two processes, also P25/H$_2$O$_2$ prevented its reactivation. Other authors (Ferro et al. 2016, Ferro et al. 2017) investigated the potential of UV/H$_2$O$_2$ process to control the potential spread of ARB into the environment. Despite of a successful inactivation of total coliforms, E. coli and antibiotic resistant counterparts, the genes bla$_{TEM}$, qnrS and tetW were still present in wastewater after 240 min of treatment, in accordance with the results of the present study.

Overall, the studied processes led to a slight reduction in the abundance of targeted genes immediately after treatment. Only bla$_{CTX,M}$, with a relatively low initial abundance, was reduced after H$_2$O$_2$ associated treatments to values below the limit of quantification. Although these types of treatment were observed to cause higher reductions in the other genes analysed, none of these ARGs were reduced to levels below LOQ. A further investigation would be necessary to assess whether disinfection driven reductions of ARGs were due the loss of the ARG (for instance plasmid loss or ARG excision), with maintenance of the host cell viability or if such reductions were due to cell inactivation. However, the observation of reactivation after 3-days storage in most of the cases, suggest that at least part of the ARG removal was due to cell inactivation and not to ARG loss. These results suggest that at least some of the bacterial cells survived these treatments, being able to reproduce during storage. However, further research on this subject is needed to withdraw safe conclusions. Regarding the apparent inconsistency between data obtained based on qPCR analyses and cultivation methods, in fact it could be expected given the
low fitness of the indicators of faecal contamination to grow in water. Moreover, it suggests that regrowth is due to other bacterial populations, as it can be confirmed below with the data based on the bacterial community analysis.

The effect of the different treatment processes on the bacterial communities was another aim of this study. Out of the 49 phyla found in freshly collected wastewater (before treatment), *Proteobacteria* (62 ± 7%) and *Bacteroidetes* (10 ± 2%) were, in average (n=8), the most abundant (Fig. 5a, t0). *Proteobacteria* comprised mainly members of the classes *Beta*-(28 ± 2%), *Gamma*-(20 ± 10%), *Alpha*-(7 ± 2%) and *Deltaproteobacteria* (5 ± 1%). Beside these groups, several other bacterial classes were detected in the freshly collected wastewater in relative abundances ranging from 2 ± 1% to 4 ± 2%; examples are “*Saprospirae*”, *Flavobacteria* and *Bacteroidia* (*Bacteroidetes*), *Clostridia* (*Firmicutes*), *Planctomycetia* (*Planctomycetes*), ZB2 (*OD1*) and *TM7-1* (*TM7*). The other identified classes had abundances < 1.3% (Fig. 5a, t0).

Regardless of the solar driven process, treatment followed by storage at room conditions led to important bacterial community rearrangements that, in general, had the same pattern. The relative abundance of the members of *Proteobacteria* was higher (p < 0.01) in the stored treated wastewater than in the freshly collected wastewater samples, whereas it was lower (p < 0.01) for the majority of members of the other phyla (Fig. 5a, t3). The lower values of the alpha-diversity indices of the stored treated wastewater samples when compared with those of freshly collected wastewater samples (Table 1) corroborate this loss of diversity and equitability. These rearrangements are well depicted in the PCoA biplot, where the bacterial communities of the freshly collected wastewater samples were separated from those treated and stored over axis PC1 (31.0%) (Fig. 6, squares and stars, respectively).

![FIGURE 4](image)

**TABLE 1**
Differences on the structure and composition of the bacterial community of the stored treated wastewater samples were mainly based on the relative abundance of Beta- and Gammaproteobacteria and Bacilli (Figs. 5a, t3 and Fig. 6, stars). The structure and composition of the bacterial communities of treated stored water was similar. Nevertheless, the relative abundance of Betaproteobacteria was higher for photolysis > P25 > P25/H$_2$O$_2$ > solar-H$_2$O$_2$ > GO-TiO$_2$, varying between 57% and 7% (Fig. 5a). Consequently, the relative abundance of Gammaproteobacteria followed a kind of inverse order (i.e., GO-TiO$_2$ > P25/H$_2$O$_2$ > P25 > solar-H$_2$O$_2$ > photolysis), varying between 61% and 25% (Fig 5a). Despite the lower values when compared with these proteobacterial classes, the relative abundance of Bacilli followed a similar order (8% and 0%) (Fig. 5a). The ability of some members of class Bacilli to survive under harsh stressful conditions through the production of resistance forms (endospores) as well as their ability to withstand oxidative stress (Battistuzzi and Hedges 2009, Mols and Abee 2011) may explain their survival upon these treatments.

**FIGURE 6**

Altogether, the results obtained suggest that the solar-driven AOPs inactivated less efficiently Beta- and/or Gammaproteobacteria or that at least some bacteria belonging to these classes have higher capacity to regrow. Curiously, at the genus level, it is possible to observe that members of the ubiquitous genus Pseudomonas were, in general, the group with the sharpest increase during storage, followed by Rheinheimera and Methilotenera (Fig. 5b). Alterations in the composition and structure of the bacterial communities leading to higher proportions of Proteobacteria in stored water than before the treatment were reported in the literature for photolysis, ozonation and photocatalytic ozonation (Becerra-Castro et al. 2016), coupled biological and photocatalysis treatments (Chen et al. 2013) and ozonation coupled to a sequencing batch biofilm reactor (Esplugas et al. 2013).
Conclusions

Among the tested solar-driven oxidation processes, photo-Fenton at circumneutral pH was the worst performing one (quite similar to photolysis), whereas the combination of P25 and H$_2$O$_2$ was the most efficient approach for the removal of organic micropollutants in the urban wastewater sampled. Regarding the biological indicators, a decrease in the abundance of total faecal coliforms and enterococci and their antibiotic resistant counterparts was found for all the processes employing H$_2$O$_2$, which was permanent after 3-days storage of the treated wastewater. P25/H$_2$O$_2$ and solar-H$_2$O$_2$ were also able to reduce the total bacterial load, assessed based on the abundance of the 16S rRNA gene. Nevertheless, the abundance of total bacterial load increased after 3-days storage to values close or higher than those verified before treatment. Similar observations were found for the genes intI1 and sulI. Hence, none of the studied processes was able to prevent bacterial reactivation, including antibiotic resistant populations. Thus, among all the studied processes, P25/H$_2$O$_2$ seemed to be that achieving the best compromise for the removal of both organic micropollutants and biological contaminants, although not able to prevent bacterial reactivation.

Interestingly, regardless of the oxidation process studied, higher relative abundance of the phylum Proteobacteria (Beta- and Gammaproteobacteria), namely of genera Pseudomonas, Rheinheimera and Methylotenera, was observed in treated wastewater after 3-days storage. Since within the phylum Proteobacteria, in particular of the classes Beta- and Gammaproteobacteria, it is possible to find diverse multidrug-resistant bacteria, the increase of this group of organisms in stored treated water may deserve further investigation. Moreover, the potential disturbance of the water bacterial communities may have relevant ecology implication and should be considered in the design of advanced oxidation technologies.
Acknowledgments

This work was financially supported by Project nº P1404290052 under the SFERA Program (EC/FP7 - Integrating Activities), Project POCI-01-0145-FEDER-006984 – Associate Laboratory LSRE-LCM (UID/EQU/50020/2013) and POCI-01-0145-FEDER-006939 (LEPABE – UID/EQU/00511/2013), funded by FEDER through COMPETE2020 - Programa Operacional Competitividade e Internacionalização (POCI) – and by national funds through FCT - Fundação para a Ciência e a Tecnologia; UID/Multi/50016/2013-CBQF and Water JPI/0001/2013 STARE, and partially co-financed by QREN, ON2, FCT and FEDER through project AIProcMat@N2020 NORTE-01-0145-FEDER-000006, NORTE-07-0162-FEDER-000050 and NORTE-01-0145-FEDER-000005 (LEPABE-2-ECO-INNOVATION), supported by North Portugal Regional Operational Program (NORTE 2020), under the Portugal 2020 Partnership Agreement, through the ERDF. NFFM, LMPM and AMTS acknowledge PD/BD/114318/2016, IF/01248/2014 and IF/01501/2013, respectively. The authors would like to acknowledge the financial support provided by COST-European Cooperation in Science and Technology, to the COST Action ES1403: New and emerging challenges and opportunities in wastewater reuse (NEREUS). Disclaimer: The content of this article is the authors' responsibility and neither COST nor any person acting on its behalf is responsible for the use, which might be made of the information contained in it.

References


Table 1. Alpha diversity indices of the wastewater samples before ($t_0$) and after 3-days storage after treatment ($t_3$) calculated based on the average of 10 rarefaction OTU tables.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Time</th>
<th>OTUs No.</th>
<th>Shannon</th>
<th>Simpson</th>
<th>PD whole tree</th>
</tr>
</thead>
<tbody>
<tr>
<td>P25</td>
<td>$t_0$</td>
<td>3023</td>
<td>7.0</td>
<td>0.95</td>
<td>125.6</td>
</tr>
<tr>
<td>P25 *</td>
<td>$t_0$</td>
<td>3181</td>
<td>8.3</td>
<td>0.99</td>
<td>158.7</td>
</tr>
<tr>
<td>P25</td>
<td>$t_3$</td>
<td>3267</td>
<td>6.6</td>
<td>0.92</td>
<td>101.8</td>
</tr>
<tr>
<td>P25 *</td>
<td>$t_3$</td>
<td>4010</td>
<td>7.3</td>
<td>0.96</td>
<td>123.8</td>
</tr>
<tr>
<td>P25 / H$_2$O$_2$</td>
<td>$t_0$</td>
<td>3353</td>
<td>7.8</td>
<td>0.98</td>
<td>156.7</td>
</tr>
<tr>
<td>P25 / H$_2$O$_2$ *</td>
<td>$t_0$</td>
<td>3586</td>
<td>8.1</td>
<td>0.98</td>
<td>164.8</td>
</tr>
<tr>
<td>P25 / H$_2$O$_2$</td>
<td>$t_3$</td>
<td>2462</td>
<td>6.1</td>
<td>0.93</td>
<td>59.4</td>
</tr>
<tr>
<td>P25 / H$_2$O$_2$ *</td>
<td>$t_3$</td>
<td>2047</td>
<td>4.9</td>
<td>0.84</td>
<td>58.7</td>
</tr>
<tr>
<td>GO-TiO$_2$</td>
<td>$t_0$</td>
<td>3425</td>
<td>8.1</td>
<td>0.98</td>
<td>161.2</td>
</tr>
<tr>
<td>GO-TiO$_2$ *</td>
<td>$t_0$</td>
<td>2687</td>
<td>7.6</td>
<td>0.97</td>
<td>136.3</td>
</tr>
<tr>
<td>GO-TiO$_2$</td>
<td>$t_3$</td>
<td>2419</td>
<td>5.6</td>
<td>0.83</td>
<td>95.5</td>
</tr>
<tr>
<td>GO-TiO$_2$ *</td>
<td>$t_3$</td>
<td>2628</td>
<td>6.5</td>
<td>0.93</td>
<td>92.7</td>
</tr>
<tr>
<td>H$_2$O$_2$</td>
<td>$t_0$</td>
<td>3161</td>
<td>7.8</td>
<td>0.98</td>
<td>155.7</td>
</tr>
<tr>
<td>H$_2$O$_2$ *</td>
<td>$t_0$</td>
<td>3567</td>
<td>8.1</td>
<td>0.98</td>
<td>166.3</td>
</tr>
<tr>
<td>H$_2$O$_2$</td>
<td>$t_3$</td>
<td>2179</td>
<td>5.2</td>
<td>0.84</td>
<td>79.4</td>
</tr>
<tr>
<td>H$_2$O$_2$ *</td>
<td>$t_3$</td>
<td>2595</td>
<td>6.3</td>
<td>0.91</td>
<td>136.2</td>
</tr>
<tr>
<td>Blank</td>
<td>$t_0$</td>
<td>3023</td>
<td>7.0</td>
<td>0.95</td>
<td>125.6</td>
</tr>
<tr>
<td>Blank *</td>
<td>$t_0$</td>
<td>3181</td>
<td>8.3</td>
<td>0.99</td>
<td>158.7</td>
</tr>
<tr>
<td>Blank</td>
<td>$t_3$</td>
<td>3487</td>
<td>6.7</td>
<td>0.94</td>
<td>112.4</td>
</tr>
<tr>
<td>Blank *</td>
<td>$t_3$</td>
<td>3511</td>
<td>6.3</td>
<td>0.91</td>
<td>111.0</td>
</tr>
</tbody>
</table>

* indicates a second independent assay.
Figure 1. Normalized concentration ($C/C_0$) of (a) CBZ, (b) SMX and (c) DFC spiked in urban wastewater as function of accumulated energy ($Q_{UV}$) using different solar-driven treatments. Except for TiO$_2$, values are the average of four (P25), three (GO-TiO$_2$, H$_2$O$_2$, Fe$^{2+}$/H$_2$O$_2$) and two (P25/H$_2$O$_2$, GO-TiO$_2$/H$_2$O$_2$, Blank) independent assays. Error bars represent standard deviations.
Figure 2. Faecal coliforms (a) and enterococci (c) and their antibiotic resistant counterparts (b, d) inactivation in urban wastewater as function of accumulated energy ($Q_{UV}$) using different solar-driven treatments. Except for TiO$_2$, values are the average of four (P25), three (GO-TiO$_2$, H$_2$O$_2$, Fe$^{2+}$/H$_2$O$_2$) and two (P25/H$_2$O$_2$, GO-TiO$_2$/H$_2$O$_2$, Blank) independent assays. Error bars represent standard deviations. DL, detection limit.
Figure 3. (a) Faecal coliforms and (c) enterococci and their (b, d) antibiotic resistant counterparts counts before, after treatment and after 3-days storage using different solar-driven treatments. Except for TiO$_2$, values are the average of four (P25), three (GO-TiO$_2$, H$_2$O$_2$, Fe$^{2+}$/H$_2$O$_2$) and two (P25/H$_2$O$_2$, GO-TiO$_2$/H$_2$O$_2$, Blank) independent assays. Error bars represent standard deviations.
Figure 4. Abundance of target genes before and after treatment, and after 3-days storage at room temperature using different solar-driven treatments: (a) 16S rRNA, (b) intI1, (c) qnrS, (d) bla_{CTX-M}, (e) sul1 and (f) bla_{TEM}. Values are the average of two independent assays. Error bars represent standard deviations.
**Figure 5.** Relative abundance of (a) classes and (b) genera before ($t_0$) and 3-days after treatment ($t_3$). *, indicates a second independent assay.
**Figure 6.** Biplot of principal coordinates analysis (PCoA) based on weighted Unifrac distances of samples before ($t_0$ - squares) and 3-days storage after treatment ($t_3$ – stars).
Highlights

• Different solar-driven advanced oxidation processes were studied at pilot-scale.
• P25/H₂O₂ showed a best compromise to remove both chemical & biological pollutants.
• P25/H₂O₂ didn’t prevent reactivation of antibiotic resistant genes in stored water.
• Beta- and Gammaproteobacteria relative abundance increased in stored treated water.