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Abstract: The release of antibiotics into the environment can result in antibiotic resistance (AR) spread, which in turn can seriously affect human health. Antibiotic resistant bacteria have been detected in different aquatic environments used as drinking water source. Water disinfection may be a possible solution to minimize AR spread but conventional processes, such as chlorination, result in the formation of dangerous disinfection by-products. In this study Advanced Oxidation Processes (AOPs), namely H2O2/UV, TiO2/UV and N-TiO2/UV, have been compared with chlorination in the inactivation of an AR Escherichia coli (E. coli) strain in surface water. TiO2 P25 and nitrogen doped TiO2 (N-TiO2), prepared by sol-gel method at two different synthesis temperatures (0 and -20°C), were investigated in heterogeneous photocatalysis experiments. Under the investigated conditions, chlorination (1.0 mg L-1) was the faster process (2.5 min) to achieve total inactivation (6 Log). Among AOPs, H2O2/UV resulted in the best inactivation rate: total inactivation (6 Log) was achieved in 45 min treatment. Total inactivation was not observed (4.5 Log), also after 120 min treatment, only for N-doped TiO2 synthesized at 0°C. Moreover, H2O2/UV and chlorination processes were evaluated in terms of cytotoxicity potential by means of 3-(4,5-dime-thylthiazol-2-yl)-2,5-diphenylte-trazolium colorimetric test on a human-derived cell line and they similarly affected HepG2 cells viability.

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***Graphical Abstract**

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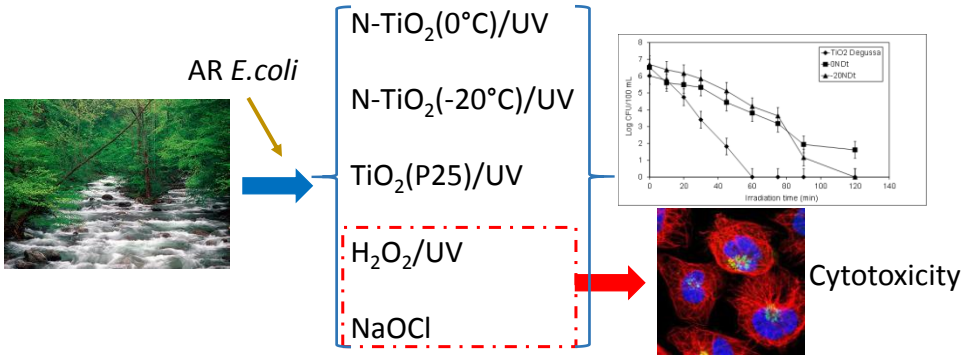

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Highlights

- All processes but N-TiO_2 (0°C synthetized) totally inactivated AR *E. coli* strain
- Chlorination was faster than AOPs in the inactivation of AR *E. coli* strain
- H_2O_2/UV was faster than TiO_2 and N-TiO_2 photocatalytic processes
- Cytotoxicity of disinfection processes evaluated by human-derived cells
- H_2O_2/UV and chlorination similarly affected human-derived cells viability

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Surface water disinfection by chlorination and advanced oxidation processes: inactivation of an antibiotic resistant *E. coli* strain and cytotoxicity evaluation.

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Abstract

The release of antibiotics into the environment can result in antibiotic resistance (AR) spread, which in turn can seriously affect human health. Antibiotic resistant bacteria have been detected in different aquatic environments used as drinking water source. Water disinfection may be a possible solution to minimize AR spread but conventional processes, such as chlorination, result in the formation of dangerous disinfection by-products. In this study Advanced Oxidation Processes (AOPs), namely H_2O_2/UV, TiO_2/UV and N-TiO_2/UV, have been compared with chlorination in the inactivation of an AR *Escherichia coli* (*E. coli*) strain in surface water. TiO_2 P25 and nitrogen doped TiO_2 (N-TiO_2), prepared by sol-gel method at two different synthesis temperatures (0 and -20°C), were investigated in heterogeneous photocatalysis experiments. Under the investigated conditions, chlorination (1.0 mg L^{-1}) was the faster process (2.5 min) to achieve total inactivation (6 Log). Among AOPs, H_2O_2/UV resulted in the best inactivation rate: total inactivation (6 Log) was achieved in 45 min treatment. Total inactivation was not observed (4.5 Log), also after 120 min treatment, only for N-doped TiO_2 synthesized at 0°C. Moreover, H_2O_2/UV and chlorination processes were evaluated in terms of cytotoxicity potential by means of 3-(4,5-dime-thylthiazol-2-yl)-2,5-diphenylte-trazolium colorimetric test on a human-derived cell line and they similarly affected HepG2 cells viability.

Keywords: antibiotic resistance, drinking water, H_2O_2/UV, photocatalysis, cytotoxicity MTT assay

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1. Introduction

Antibiotic resistance (AR) is a threat for human and animal health worldwide and key measures are required to reduce AR spread in the environment. Antibiotics resistant bacteria (ARB), antibiotic resistant genes (ARG) and antibiotics have been found in various aquatic environments including rivers and lakes (Storteboom et al., 2010; Young et al., 2013), drinking water systems (Bergeron et al., 2015; Flores Ribeiro et al., 2014; Xi et al., 2009), coastal bathing water (Leonard et al., 2015) and wastewater (Rizzo et al., 2013; Schwartz et al., 2003). As such, ARB and ARGs are recognized as emerging pollutants (Pruden et al., 2006). In order to reduce AR spread in the environment as well as the risk for human health, water and wastewater should be disinfected before use and reuse/disposal, respectively. Chlorination and UV radiation are among the most widely used disinfection processes but they may be not effective in controlling AR spread (Fiorentino et al., 2015; Guo et al., 2013; Huang et al., 2013). Moreover, chlorine reacts with organic matter and other precursors to form regulated and emerging carcinogenic disinfection by-products (DBPs) (Richardson et al., 2007). Epidemiological studies have shown that consumption or exposure to DBPs concentrations above the maximum containment level in water can be associated with problems of liver, kidney, the central nervous system and increased risks of bladder, and colorectal cancers (Bull et al., 2011; Rahman et al., 2010; Villanueva et al., 2007). Advanced Oxidation Processes (AOP) (e.g., Fenton, photo-Fenton, TiO_2/UV, UV/O_3, UV/H_2O_2, etc.) have been investigated as possible alternative to conventional disinfection processes, and photo-driven AOPs can be also operated with natural sunlight (Malato et al., 2009). Among AOPs, TiO_2 photocatalysis has recently emerged as an interesting water disinfection option for solar applications (Fiorentino et al., 2015), but due to TiO_2 capacity to absorb only about 4% of the solar irradiation, current challenge is to investigate new photocatalysts modified to broad the absorption of TiO_2 toward the visible region. TiO_2 doping by non-metallic species such as N is an interesting option to improve process efficiency (Rizzo et al., 2014c). Our group investigated the effect of different AOPs (among which UV/H_2O_2) on ARB in urban wastewater (Fiorentino et al., 2015), but there is a lack of information about the effect of these processes in terms of ARB inactivation and toxicity in drinking water. As matter of fact, if not correctly operated AOPs can result in the formation of dangerous oxidation intermediates (Rizzo, 2011) and increase subsequent formation of DBPs too. For instance, TiO_2 photocatalysis exacerbated the production of total

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trihalomethanes (TTHMs) and haloacetic acids (HAA) beyond initial levels when operated at low-energy (<5 kW h m^3) (Mayer et al., 2014). Low-energy photocatalysis can result in an incomplete oxidation, wherein larger, more aromatic, humic organic compounds are broken into smaller molecular weight, less aromatic and less humic moieties, which have considerable potential to produce DBPs. More complete mineralization of DBP precursors was obtained using extended photocatalysis (80 - 160 kW h m^3), which substantially decreased DBP precursors as well as TTHM and HAA concentrations. Moreover, the impact of UV/H_2O_2 pretreatment on subsequent TTHM and HAA formation can increase the respective concentrations at low and moderate AOP doses (e.g., UV doses in the order of up to 1000 mJ cm^{-2}) after chlorination (Dotson et al., 2010; Kleiser and Frimmel, 2000), while higher AOP doses (UV of 3500-5000 mJ cm^{-2}) can decrease THM and HAA formation (Liu et al., 2002; Toor and Mohseni, 2007). As for the regulation of DBPs, only a small number of genotoxic and cytotoxic DBPs are regulated and therefore many DBPs with similar consequences remain unregulated (Plewa et al., 2010; Richardson et al., 2007). Moreover, only a few of the 600 DBPs identified (Richardson et al., 2007) have been analyzed for their cytotoxic and genotoxic potential and impact on health issues (Muellner et al., 2007; Plewa et al., 2010). Typically, the evaluation of DBPs genotoxic and cytotoxic effects is compound specific (Ali et al., 2014; Escobar-Hoyos et al., 2013; Plewa et al., 2010) and only a few studies addressed the effect of the disinfection process on genotoxicity and cytotoxicity of real water matrix (Magdeburg et al., 2014). In particular, the simultaneous effect of AOPs on ARB inactivation and possible formation of toxic oxidation intermediates during drinking water disinfection has not yet been investigated nor compared with a wide used disinfection process such as chlorination.

Therefore, the aim of the present work was to compare chlorination and AOPs (namely, H_2O_2/UV, TiO_2/UV and N-TiO_2/UV) in surface water disinfection in terms of AR *E. coli* strain inactivation and cytotoxicity. Subsequently, the cytotoxicity of DBPs originated by disinfection with chlorine and H_2O_2/UV was evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylte-trazolium (MTT) colorimetric test on a human-derived cell line.

2. Materials and methods

2.1. Surface water samples

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Surface water samples were collected from Tusciano river (37 km, 300 L/s flow rate, 42 km² watershed) in the province of Salerno (Southern Italy). Samples were collected in amber glass vials (1 L) and were characterized for some parameters which resulted in the following average values: pH (8.3), BOD_5 (2.0 mg L^{-1}), DOC (3.5 mg. L^{-1}), *E. co*li (5 x 10^1 CFU/100 mL).

2.2. Selection and identification of AR E. coli *strain*

AR *E. coli* strain was selected from surface water sample taken from Tusciano river. 50 mL samples were filtered through a 0.45 μm pore size cellulose nitrate membranes (Millipore, MA, USA). The filtered samples, were incubated (24 h at 44 °C) on Tryptone Bile X-Glucuoronide (TBX, Oxoid Basingstoke, UK) supplemented with a mixture of three antibiotics (2 mg L^{-1} ciprofloxacin (CPX), 16 mg L^{-1} of ampicillin (AMP) and 8 mg L^{-1} tetracycline (TET)) according to a previous work (Ferro et al., 2015).

2.3. Sample preparation and inoculum

Disinfection experiments were carried out on 500 mL surface water samples. Water samples were first autoclaved at 120 °C for 15 min to avoid any potential interference with the selected AR *E. coli*. Then, autoclaved water sample was inoculated with the selected strain. Briefly, some colonies were unfrozen and reactivated by streaking on selective culture media (Chromo-Cult Coliform agar for AR *E. coli*) and, then, incubated at 37 °C for 24 h. Two colonies from each plate were inoculated into 14 mL sterile Luria−Bertani broth (LB, Sigma-Aldrich, Italy) and incubated at 37 °C for 24 h by constant agitation in a rotator shaker to obtain a stationary phase culture. Cells were harvested by centrifugation at 3000 rpm for 10 min and the pellet was resuspended in 14 mL of phosphate buffer saline (PBS, Oxoid), yielding a final concentration of ca. 10^7 colony forming unit (CFU) mL^{-1}. Inoculated water sample was kept under bland agitation for five min in absence of light before disinfection experiment started.

2.4. AOPs disinfection experiments

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Two different AOPs were evaluated: (i) homogenous photocatalytic process implementing the combination between H_2O_2 and UV irradiation and (ii) heterogeneous photocatalytic process using TiO_2 semiconductor and UV irradiation. The photocatalytic tests were carried out in a 1 L cylindrical glass reactor (14 cm diameter) filled in with 500 mL of water (5.0 cm water height). In order to keep a constant temperature of 25 °C, the reactor was placed in a water bath. The water solution was magnetically stirred during the experiment. The experiments were conducted in a box equipped with a wide spectrum 250 W lamp (Procomat, Florence - Italy), set at 33 cm from the surface of the water level in the vessel. A spectrometer model HR-2000 from Ocean Optics (Florida, USA), equipped with cosine corrector with Spectralon diffusing material, was used to measure irradiance spectra of UV lamp (Figure 1). Control experiments with H_2O_2, TiO_2 and UV radiation as standalone processes were carried out. In heterogeneous photocatalytic experiments two photocatalysts were investigated: (i) commercially available TiO_2 Degussa P25 and (ii) nitrogen doped TiO_2 (N-TiO_2) prepared by sol-gel method at two different synthesis temperatures (0 and -20°C).

Figure 1

2.4.1 H_2O_2/UV experiments

H_2O_2 at 30 wt% (Titolchimica, Italy) was used as received and diluted into the reactor to achieve an H_2O_2 initial dose of 0.5 mmol L^{-1}; the working solution was stirred for three minutes in the dark to ensure homogenization. H_2O_2 concentration was measured by a colorimetric method based on the use of titanium (IV) oxysulfate (Sigma-Aldrich), which forms a stable yellow complex with H_2O_2 detected by absorbance measurements at 410 nm. Absorbance was measured using a spectrophotometer (PerkinElmer, USA) and was linearly correlated with H_2O_2 concentration in the range 0.1-100 mg L^{-1}. Catalase was added to water samples before analysis to eliminate residual H_2O_2; specifically, 1 mL sample was mixed with 20 µL of 2300 U mg^{-1} bovine liver catalase at 0.1 g L^{-1} (Sigma-Aldrich).

2.4.2 Photocatalytic experiments with TiO_2 (Degussa P25)

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The experiments were performed with TiO_2 (Degussa P25 - Evonik Corporation, Germany) powder used as received from the manufacturer. The photocatalyst was added to the autoclaved surface water sample and agitated for 5 minutes. The TiO_2 concentration was fixed at 0.05 g L^{-1}, according to the results achieved in previous disinfection experiments (Rizzo et al., 2014a). Finally, inoculum was added to the sample and photocatalytic experiment was started.

2.4.3. Photocatalysts preparation and photocatalytic experiments with N-TiO_2

The titanium dioxide photocatalyst doped with nitrogen (N-TiO_2) was prepared by sol-gel method at two different synthesis temperatures (0 and -20°C), according to the method described by (Vaiano et al., 2015). Briefly, Triton X-100 (nonionic surfactant, Sigma–Aldrich) was used as dispersant agent. Triton X-100 was dissolved in isopropyl alcohol (99.8 wt%, Sigma–Aldrich) and the pH of solution was adjusted with nitric acid (65 wt%, Carlo Erba) until to reach a value of about 2. Then, titanium (IV) isopropoxide (TIP, 97%,Sigma–Aldrich) was added to the mixture as precursor. A cryostat bath was used to control and monitor the temperature during the sol–gel synthesis. Once the solution reached the requested temperature, an ammonia aqueous solution (30 wt%, Sigma–Aldrich) was added as nitrogen precursor, while the water was able to induce the hydrolysis reaction with TIP. The obtained precipitated was then calcined for 30 min at 450°C. The molar ratio N/Ti was equal to 18.6 and corresponds to an optimized catalyst formulation found in a previous work (Sannino et al., 2013). The N-TiO_2 samples resulted in a yellow appearance and they were named xNDt were x is the synthesis temperature. Both samples presented an energy band-gap of 2.5 eV. The specific surface area (SSA) was 98 and 147 m^2/g for 0NDt and -20NDt, respectively. The increase of SSA with the decrease of synthesis temperature induced a decrease of average crystallites size from 9 to 6 nm (Vaiano et al., 2015). The N-TiO_2 concentration in the apparatus for photocatalytic tests was set at 0.05 g L^{-1}

2.5. Chlorination experiments

The chlorination experiments were carried out with the same water sample taken from Tusciano river (0.5 L) after autoclaving and *E. coli* strain inoculation. Sodium hypochlorite solution (1 mg L^{-1}) was added to the reactor (time “0” started just after

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sodium hypochlorite addition), the sample was continuously stirred with a magnetic stirrer, and water pH was not adjusted during the experiment. Sodium thiosulfate solution (1.5%) was added to water samples to remove the residual chlorine before microbiological analysis. The residual chlorine was measured by colorimetric method by portable HACH instrument. Chlorination experiments finalized to cytotoxicity assays were extended to 420 min treatment time to take into account the formation of chlorination by products can take some hours to reach the maximum concentration, depending on initial chlorine dose and water oxidant demand (Rizzo et al., 2007).

2.6 Bacterial count

Water samples were regularly collected and analyzed for bacterial count. Aliquots of starting solution and serial dilutions were plated onto TBX agar culture medium and incubated at 37 °C for 24 h. Counting was performed in triplicate and the mean values and standard deviations were represented as CFU 100 mL^{-1}.

2.7 Cell culture

HepG2 cells were obtained from Interlab Cell Line Collection (Centro di Biotecnologie Avanzate, Genoa, Italy). Cells were cultured in 100x10 mm Petri dishes containing Essential's modified Eagle's medium supplemented with 10% (v/v) foetal bovine serum, 1% (v/v) non-essential amino acids, 0.2 mM L-glutamine, 50 units mL^{-1} penicillin and 50 µg mL^{-1} streptomycin (Sigma-Aldrich). Cells were maintained at 37° C in a 5% CO_2, 95% air-humidified atmosphere and passaged twice a week.

2. 8 MTT cytotoxicity assay

Water samples taken from disinfection experiments were prepared in dimethylsulfoxide (DMSO) (Sigma–Aldrich). For each treatment, samples were diluted (1:2000) in complete cell culture medium, to achieve 0.05% DMSO concentration in the culture medium. Cells were seeded at the density of $9.0x10^4$ cm^{-2} in 96-well plates and cultured for 24 h. Then growth medium was removed and 100 µL of diluted samples were added to each well. Cells were incubated for further 24 h, before the cell viability MTT assay was performed. MTT assay is based on the enzymatic conversion of MTT (Sigma-

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Aldrich) by dehydrogenase enzymes in intact mitochondria of living cells (Mosmann, 1983). After incubation, MTT solution was added to the cell medium (final concentration 0.125 mg mL^{-1}) and incubated for 1 h at 37 °C to allow MTT to be metabolized. The resulting formazan crystals were dissolved in DMSO and absorbances were measured at 595 nm (reference filter 655 nm) using a microplate reader (Bio-Rad Laboratories, Milan, Italy). As control autoclaved river water samples were supplemented with catalase (as control for samples derived from H_2O_2/UV experiments) or with sodium thiosulfate, $Na_2S_2O_3$ (Sigma-Aldrich) (as control for samples derived from chlorination experiments). Cell viability was determined by comparing the optical density OD of the wells containing cells exposed to disinfected water samples and OD of the wells containing cells exposed to control samples. Data were plotted as mean ± SD from five independent experiments, each performed in triplicates. Statistical analysis was performed by using the Student's t test and differences were considered to be statistically significant at $p < 0.05$.

3. Results and discussion

3.1. *Inactivation of* AR E. coli *strain by H_2O_2/UV process*

Figure 2 shows inactivation results of AR *E. coli* strain by H_2O_2/UV disinfection process and related control with UV and H_2O_2 as standalone processes, respectively. Control test with H_2O_2 did not show any significant inactivation until 75 min treatment; then a 1 Log inactivation was observed after 90 min. UV radiation treatment did not affect initial *E. coli* density until 30 min irradiation, but after 75 min a 4 Log inactivation was observed.

Figure 2

Inactivation rate observed during UV radiation control test can be explained by the lamp emission spectrum (mainly in the range 340-450 nm, Figure 1), through the damage caused to intracellular chromophores due to radiation absorption. Although the UV-A wavelengths are not sufficiently energetic to directly modify DNA bases, they play an important role in the formation of reactive oxygen species (ROS) in water which can

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damage DNA (McGuigan et al., 2012). Unlike control tests, H_2O_2/UV process resulted in a total inactivation of AR *E.coli* (6 Log) after 45 min treatment. AOPs, such as H_2O_2/UV, result in the formation of ROS which can inactivate a wide range of microorganisms (Malato et al., 2009; Rizzo et al., 2014b; Robertson et al., 2012). Among ROS, hydroxyl radicals ($^{\bullet}OH$) can effectively inactivate *E. coli*; an excellent linear correlation between $^{\bullet}OH$ and the rate of *E. coli* inactivation was observed in a previous work (Cho et al., 2004). The inactivation rates observed in the present work are in agreement with results available in scientific literature. In particular, the inactivation rate of an AR *E. coli* strain suspended in urban wastewater treated by H_2O_2/sunlight increased as H_2O_2 dose was increased (10, 20, 50 mg/L); total inactivation (initial cell density 10^6 CFU/mL) was observed at 30, 18 and 8 kJ L^{-1}, respectively (Fiorentino et al., 2015); 240 min treatment was necessary to achieve total *E. coli* inactivation (5 Log) by H_2O_2/UV process using SUNTEST CPS solar chamber (emission spectrum 320-800 nm, light intensity of 500 W m^{-2}) and 25 mg H_2O_2 L^{-1} (Rodriguez-Chueca et al., 2015).

3.2. *Inactivation of AR* E. coli *by heterogeneous photocatalysis*

In heterogeneous photacatalysis disinfection experiments, total inactivation was achieved within 60 and 120 min of irradiation for TiO_2 Degussa and -20NDt, respectively (Figure 3).

Figure 3

The obtained inactivation behavior is in agreement with previous results about *E. coli* inactivation by photocatalysis using P25 and TiO_2 doped with nitrogen and sulphur (Pathakoti et al., 2013). The authors observed that P25 has an inactivation rate higher than doped-TiO_2 under solar simulated light, whereas, under visible light, doped-TiO_2 sample showed photocatalytic performances better than P25. Considering these observations and that the maximum emission intensity of the lamp used in the present work was in the UV region (350 nm, Figure 1), the lower photocatalytic activity of -20NDt with respect to TiO_2 Degussa was possibly due to the lower emission of the lamp in the visible region (400-460 nm). Moreover, N-TiO_2 photocatalyst prepared by sol-gel

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method at -20°C synthesis temperature (-20NDt) was more effective than that one synthetized at 0°C (0NDt): total inactivation (6.5 Log) was observed after 120 min treatment with -20NDt while 4.5 Log inactivation was observed for 0NDt photocatalyst after the same treatment time. The better disinfection performance observed with -20NDt is possibly due to its higher SSA (147 $m^2 g^{-1}$) with respect to that one of 0NDt (98 $m^2 g^{-1}$) (Vaiano et al., 2015). The increase in SSA should help in increasing the -20NDt exposure to light which in turn would increases the photocatalytic inactivation rate.

In spite of the different aqueous matrix investigated, the inactivation rate observed for TiO_2 P25 is also in agreement with our previous work where the effect of different light sources on photocatalytic (TiO_2 P25, 0.05 g L^{-1}) inactivation of an AR *E. coli* strain in urban wastewater (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 mS cm^{-1}.) was evaluated (Rizzo et al., 2014a); accordingly, total inactivation (approximately 7 Log) was observed after 60 min treatment.

3.3. Chlorine test

Initial chlorine dose as high as 1.0 mg L^{-1} was sufficient to achieve a total inactivation of AR *E. coli* (6 Log) after 2.5 min contact time. When the same experiment was carried out with urban wastewater inoculated with an AR *E. coli* strain, total inactivation (approximately 5 Log) was observed after 15 min contact time for the same initial chlorine dose (Fiorentino et al., 2015). Basically, the difference between the results may be due to the respective aqueous matrices investigated; urban wastewater are typically characterized by a significantly higher concentration of oxidant consuming compounds (oxidant demand), such as inorganic N compounds. Accordingly, as contact time increases chlorine is consumed and consequently it is no longer available for bacterial inactivation (Zhang et al., 2015). Chlorination process can be effective in the inactivation of ARB but does not affect AR of survived cells (Rizzo et al., 2012). In particular, chlorination process can affect the microbial structure, enrich ARB (Shi et al., 2013) as well as increase the selection of ARB in drinking water (Pang et al., 2015).

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3.4. Comparative cytotoxic evaluation between chlorination and H_2O_2/UV processes

Finally, H_2O_2/UV process was chosen as model AOP to be compared with chlorination process in terms of cytotoxic effects. The cytotoxicity of disinfected water samples was tested by the colorimetric MTT assay. For this assay HepG2 cell line was used, a highly differentiated human hepatoma cell line intensively used for toxicity evaluation of pure compounds and of chemicals in complex mixtures (Mersch-Sundermann et al., 2004). Control tests with autoclaved river water samples supplemented with catalase (as control for samples derived from H_2O_2/UV experiments) and with sodium thiosulfate (as control for samples derived from chlorination experiments), used to eliminate residual H_2O_2 and chlorine, respectively, did not significantly reduce cell viability. After treating cells for 24 h with a 1:2000 solution (prepared in culture medium) of samples obtained from both H_2O_2/UV and chlorination processes, a slight and comparable cytotoxic effect was observed (Figures 4 and 5). Interestingly, the only sample which did not result in a significant difference in cell viability compared to control in H_2O_2/UV disinfection experiments was that one taken after 120 min treatment (Figure 4).

Figure 4

Possibly, shorter treatment time results in the formation of oxidation intermediates with higher cytotoxicity compared to untreated sample (decreasing trend in cell viability in the early 30 min); further increasing treatment time (up to 120 min) results in a progressive disappearance of the oxidation intermediates (Klamerth et al., 2010; Rizzo et al., 2009; Selcuk et al., 2007). DBPs forming during chlorination process can take some hours to reach the maximum concentration, depending on initial chlorine dose and water oxidant demand. Accordingly, chlorination experiments for cytotoxicity evaluation were extended up to 420 min (Figure 5). Although this is not a realistic disinfection contact time (typically in the range 30-60 min for chlorination process), it was useful to better understand the effect of chlorination process on treated water cytotoxicity.

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Figure 5

The results achieved are quite consistent with the matter that cytotoxicity is expected to increase (cell viability decrease) as treatment time was increased because in parallel a higher concentration of chlorination by products is expected (Rizzo et al., 2007).

4. Conclusion

Chlorination process was really fast in the inactivation of AR *E. coli*; 2.5 min contact time and 1.0 mg L^{-1} of chlorine dose were sufficient to achieve a total removal (6 Log). H_2O_2/UV (0.5 mmol H_2O_2 L^{-1}) process was found to be faster than heterogeneous photocatalytic process; total inactivation of AR *E.coli* (6 Log) was achieved in 45 min treatment compared to 60 min treatment necessary during TiO_2 (P25, 0.05 g L^{-1}) photocatalysis experiments. The higher SSA of -20NDt photocatalyst possibly explain the best disinfection performance (total inactivation (6.5 Log) was observed after 120 min treatment) compared to 0NDt (4.5 Log inactivation in the same treatment time). The best disinfection time for H_2O_2/UV process (45 min) did not match the best condition in terms of cytotoxicity (120 min).

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References

Ali A, Kurzawa-Zegota M, Najafzadeh M, Gopalan RC, Plewa MJ, Anderson D., 2014. Effect of drinking water disinfection by-products in human peripheral blood lymphocytes and sperm. Mutat. Res., Fundam. Mol. Mech. Mutagen. 770, 136-143.

Bergeron S, Boopathy R, Nathaniel R, Corbin A, LaFleur G., 2015. Presence of antibiotic resistant bacteria and antibiotic resistance genes in raw source water and treated drinking water. Int. Biodeterior. Biodegrad. 102, 370-374.

Bull RJ, Reckhow DA, Li X, Humpage AR, Joll C, Hrudey SE., 2011. Potential carcinogenic hazards of non-regulated disinfection by-products: Haloquinones, halo-cyclopentene and cyclohexene derivatives, N-halamines, halonitriles, and heterocyclic amines. Toxicology. 286, 1-19.

Cho M, Chung H, Choi W, Yoon J., 2004. Linear correlation between inactivation of E. coli and OH radical concentration in TiO2 photocatalytic disinfection. Water Res. 38, 1069-1077.

Dotson AD, Keen VS, Metz D, Linden KG., 2010. UV/H2O2 treatment of drinking water increases post-chlorination DBP formation. Water Res. 44, 3703-3713.

Escobar-Hoyos LF, Hoyos-Giraldo LS, Londono-Velasco E, Reyes-Carvajal I, 2013. Saavedra-Trujillo D, Carvajal-Varona S, et al. Genotoxic and clastogenic effects of monohaloacetic acid drinking water disinfection by-products in primary human lymphocytes. Water Res. 47, 3282-3290.

Ferro G, Fiorentino A, Alferez MC, Polo-López MI, Rizzo L, Fernández-Ibáñez P., 2015. Urban wastewater disinfection for agricultural reuse: Effect of solar driven AOPs in the inactivation of a multidrug resistant E. coli strain. Applied Catalysis B: Environmental 178, 65-73.

Fiorentino A, Ferro G, Alferez MC, Polo-Lopez MI, Fernandez-Ibanez P, Rizzo L., 2015. Inactivation and regrowth of multidrug resistant bacteria in urban wastewater after disinfection by solar-driven and chlorination processes. J. Photochem. Photobiol., B. 148, 43-50.

Flores Ribeiro A, Bodilis J, Alonso L, Buquet S, Feuilloley M, Dupont J-P, et al. 2014. Occurrence of multi-antibiotic resistant Pseudomonas spp. in drinking water produced from karstic hydrosystems. Sci. Total Environ. 490, 370-378.

Guo M-T, Yuan Q-B, Yang J., 2013. Ultraviolet reduction of erythromycin and tetracycline resistant heterotrophic bacteria and their resistance genes in municipal wastewater. Chemosphere. 93, 2864-2868.

Huang J-J, Hu H-Y, Wu Y-H, Wei B, Lu Y., 2013. Effect of chlorination and ultraviolet disinfection on tetA-mediated tetracycline resistance of Escherichia coli. Chemosphere. 90, 2247-2253.

Klamerth N, Rizzo L, Malato S, Maldonado MI, Agueera A, Fernandez-Alba AR., 2010. Degradation of fifteen emerging contaminants at μgL-1 initial concentrations by mild solar photo-Fenton in MWTP effluents. Water Res. 44, 545-554.

Kleiser G, Frimmel FH., 2000. Removal of precursors for disinfection by-products (DBPs) - differences between ozone- and OH-radical-induced oxidation. Sci. Total Environ. 256, 1-9.

Leonard AFC, Zhang L, Balfour AJ, Garside R, Gaze WH., 2015. Human recreational exposure to antibiotic resistant bacteria in coastal bathing waters. Environ. Int. 82, 92-100.

Liu W, Andrews SA, Bolton JR, Linden KG, Sharpless C, Stefan M., 2002. Comparison of disinfection byproduct (DBP) formation from different UV technologies at bench scale. Water Sci. Technol. Water Supply. 2, 515-521.

Magdeburg A, Stalter D, Schluesener M, Ternes T, Oehlmann J., 2014. Evaluating the efficiency of advanced wastewater treatment: Target analysis of organic contaminants and (geno-)toxicity assessment tell a different story. Water Res. 50, 35-47.

Malato S, Fernandez-Ibanez P, Maldonado MI, Blanco J, Gernjak W., 2009. Decontamination and disinfection of water by solar photocatalysis: Recent overview and trends. Catal. Today. 147, 1-59.

Mayer BK, Daugherty E, Abbaszadegan M., 2014. Disinfection byproduct formation resulting from settled, filtered, and finished water treated by titanium dioxide photocatalysis. Chemosphere. 117, 72-78.

McGuigan KG, Conroy RM, Mosler H-J, du Preez M, Ubomba-Jaswa E, Fernandez-Ibanez P., 2012. Solar water disinfection (SODIS): A review from bench-top to roof-top. J. Hazard. Mater. 235-236, 29-46.

Mersch-Sundermann V, Knasmuller S, Wu X-j, Darroudi F, Kassie F., 2004.Use of a human-derived liver cell line for the detection of cytoprotective, antigenotoxic and cogenotoxic agents. Toxicology. 198, 329-340.

Mosmann T., 1983.Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 65, 55-63.

Muellner MG, Wagner ED, McCalla K, Richardson SD, Woo Y-T, Plewa MJ., 2007. Haloacetonitriles vs. Regulated Haloacetic Acids: Are Nitrogen-Containing DBPs More Toxic? Environ. Sci. Technol. 41, 645-651.

Pang Y, Huang J, Xi J, Hu H, Zhu Y., 2015. Effect of ultraviolet irradiation and chlorination on ampicillin-resistant Escherichia coli and its ampicillin resistance gene. Front. Environ. Sci. Eng.: Ahead of Print.

Pathakoti K, Morrow S, Han C, Pelaez M, He X, Dionysiou DD, et al., 2013. Photoinactivation of Escherichia coli by Sulfur-Doped and Nitrogen-Fluorine-Codoped TiO_2 Nanoparticles under Solar Simulated Light and Visible Light Irradiation. Environ. Sci. Technol. 47, 9988-9996.

Plewa MJ, Simmons JE, Richardson SD, Wagner ED., 2010.Mammalian cell cytotoxicity and genotoxicity of the haloacetic acids, a major class of drinking water disinfection by-products. Environ. Mol. Mutagen. 51, 871-878.

Pruden A, Pei R, Storteboom H, Carlson KH., 2006. Antibiotic Resistance Genes as Emerging Contaminants: Studies in Northern Colorado. Environ. Sci. Technol. 40, 7445-7450.

Rahman MB, Driscoll T, Cowie C, Armstrong BK., 2010. Disinfection by-products in drinking water and colorectal cancer: a meta-analysis. Int J Epidemiol.39, 733-45.

Richardson SD, Plewa MJ, Wagner ED, Schoeny R, DeMarini DM., 2007.Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: A review and roadmap for research. Mutat. Res., Rev. Mutat. Res. 636, 178-242.

Rizzo L., 2011. Bioassays as a tool for evaluating advanced oxidation processes in water and wastewater treatment. Water Res. 45, 4311-4340.

Rizzo L, Della Sala A, Fiorentino A, Li Puma G., 2014a. Disinfection of urban wastewater by solar driven and UV lamp - TiO_2 photocatalysis: Effect on a multi drug resistant Escherichia coli strain. Water Res. 53, 145-152.

Rizzo L, Ferro G, Manaia CM., 2014b. Wastewater disinfection by solar heterogeneous photocatalysis: effect on tetracycline resistant/sensitive Enterococcus strains. Global NEST J. 16 (8),457-464.

Rizzo L, Fiorentino A, Anselmo A., 2012. Effect of solar radiation on multidrug resistant E. coli strains and antibiotic mixture photodegradation in wastewater polluted stream. Sci. Total Environ. 427-428, 263-268.

Rizzo L, Manaia C, Merlin C, Schwartz T, Dagot C, Ploy MC, et al., 2013.Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: A review. Sci. Total Environ. 447, 345-360.

Rizzo L, Meric S, Guida M, Kassinos D., Belgiorno V., 2009. Heterogeneous photocatalytic degradation kinetics and detoxification of an urban wastewater treatment plant effluent contaminated with pharmaceuticals. Water Res. 43, 4070-4078.

Rizzo L, Nikolaou A, Andrzejewski P., 2007. Disinfection of drinking waters and DBPs formation: Chlorination. Control of Disinfection By-Products in Drinking Water Systems, Chapter 1.1 - Chlorination, 3-28.

Rizzo L, Sannino D, Vaiano V, Sacco O, Scarpa A, Pietrogiacomi D., 2014c. Effect of solar simulated N-doped TiO_2 photocatalysis on inactivation and antibiotic resistance of E. coli strain in biologically treated urban wastewater. Appl. Catal., B. 144, 369-378.

Robertson PKJ, Robertson JMC, Bahnemann DW., 2012.Removal of microorganisms and their chemical metabolites from water using semiconductor photocatalysis. J. Hazard. Mater. 211-212, 161-171.

Rodriguez-Chueca J, Ormad MP, Mosteo R, Ovelleiro JL., 2015.Kinetic modeling of Escherichia coli and Enterococcus sp. inactivation in wastewater treatment by photo-Fenton and H_2O_2/UV-vis processes. Chem. Eng. Sci. 138, 730-740.

Sannino D, Vaiano V, Sacco O, Ciambelli P., 2013. Mathematical modelling of photocatalytic degradation of methylene blue under visible light irradiation. Journal of Environmental Chemical Engineering. 1, 56-60.

Schwartz T, Kohnen W, Jansen B, Obst U., 2003. Detection of antibiotic-resistant bacteria and their resistance genes in wastewater, surface water, and drinking water biofilms. FEMS Microbiol. Ecol. 43, 325-335.

Selcuk H, Rizzo L, Nikolaou AN, Meric S, Belgiorno V, Bekbolet M., 2007. DBPs formation and toxicity monitoring in different origin water treated by ozone and alum/PAC coagulation. Desalination. 210, 31-43.

Shi P, Jia S, Zhang X-X, Zhang T, Cheng S, Li A., 2013. Metagenomic insights into chlorination effects on microbial antibiotic resistance in drinking water. Water Res. 47, 111-120.

Storteboom H, Arabi M, Davis JG, Crimi B, Pruden A., 2010.Identification of Antibiotic-Resistance-Gene Molecular Signatures Suitable as Tracers of Pristine River, Urban, and Agricultural Sources. Environ. Sci. Technol. 44, 1947-1953.

Toor R, Mohseni M., 2007.UV-H_2O_2 based AOP and its integration with biological activated carbon treatment for DBP reduction in drinking water. Chemosphere. 66, 2087-2095.

Vaiano V, Sacco O, Sannino D, Ciambelli P., B 2015.Nanostructured N-doped TiO_2 coated on glass spheres for the photocatalytic removal of organic dyes under UV or visible light irradiation. Appl. Catal. 170-171, 153-161.

Villanueva CM, Cantor KP, Grimalt JO, Malats N, Silverman D, Tardon A, et al., 2007.Bladder cancer and exposure to water disinfection by-products through ingestion, bathing, showering, and swimming in pools. Am J Epidemiol. 165, 148-56.

Xi C, Zhang Y, Marrs CF, Ye W, Simon C, Foxman B, et al., 2009. Prevalence of antibiotic resistance in drinking water treatment and distribution systems. Appl. Environ. Microbiol. 75, 5714-5718.

Young S, Juhl A, O'Mullan GD., 2013.Antibiotic-resistant bacteria in the Hudson River Estuary linked to wet weather sewage contamination. J Water Health. 11, 297-310.

Zhang Y, Zhuang Y, Geng J, Ren H, Zhang Y, Ding L, et al., 2015.Inactivation of antibiotic resistance genes in municipal wastewater effluent by chlorination and sequential UV/chlorination disinfection. Science of the Total Environment. 512-513, 125-132.

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Figures captions

Figure 1: light intensity spectrum of 250 W lamp used in photocatalytic experiments.

Figure 2: inactivation of AR *E. coli* by H_2O_2/UV process and control tests (H_2O_2 and UV radiation as standalone processes, respectively; H_2O_2 concentration of 0.5 mmol L^{-1}).

Figure 3: inactivation of AR *E. coli* by heterogeneous photocatalysis with different catalysts (0.05 g L^{-1}): commercially available TiO_2 Degussa P25 and nitrogen doped TiO_2 prepared by sol-gel method at two different synthesis temperatures 0°C (0NDt) and -20°C (-20NDt).

Figure 4: normalized cell viability of HepG2 cells after 24 h exposure to water samples treated by H_2O_2/UV process (H_2O_2 initial dose: 0.5 mmol L^{-1}); *$p<0.05$ vs. the respective control.

Figure 5: normalized cell viability of HepG2 cells after 24 h exposure to water samples treated by chlorine (initial does 1 mg L^{-1}); *$p<0.05$ vs. the respective control.

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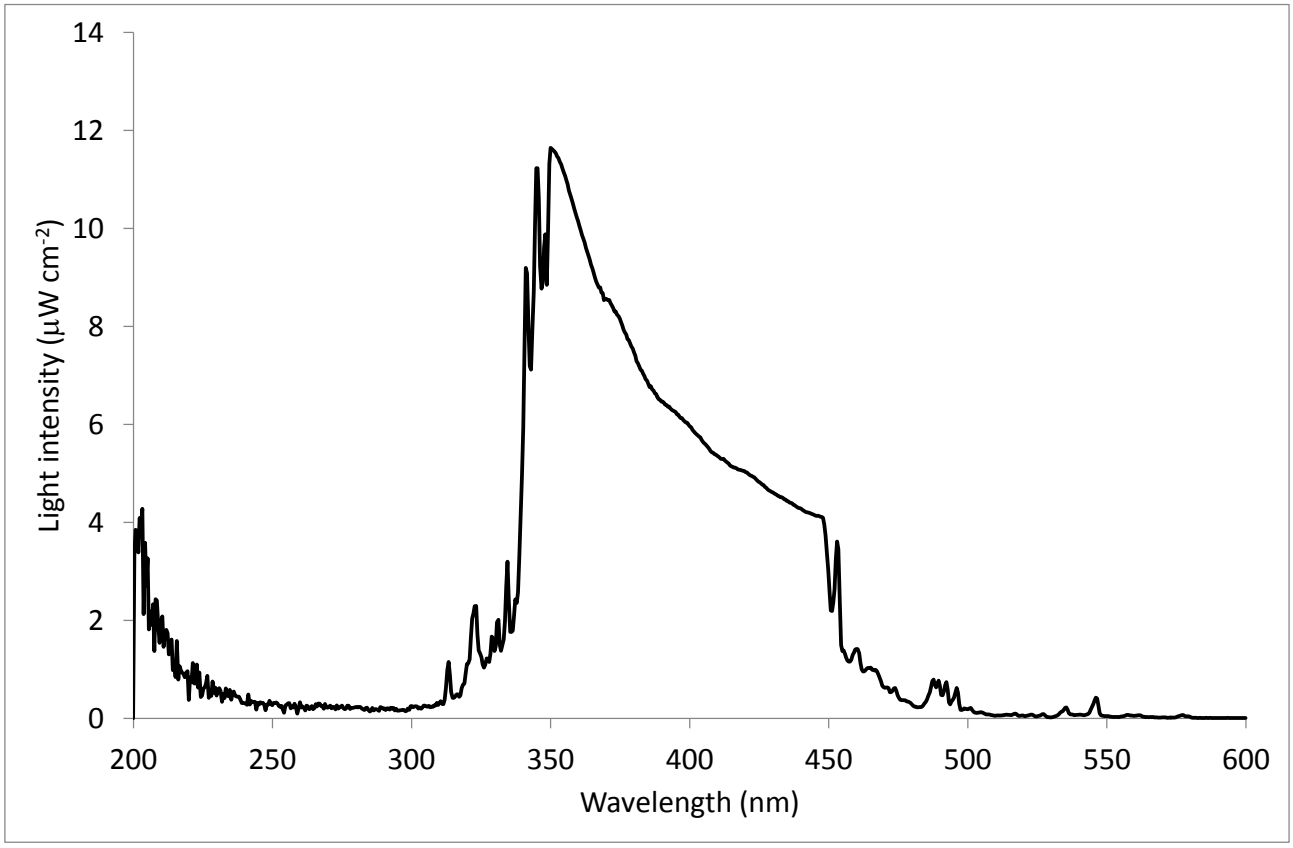

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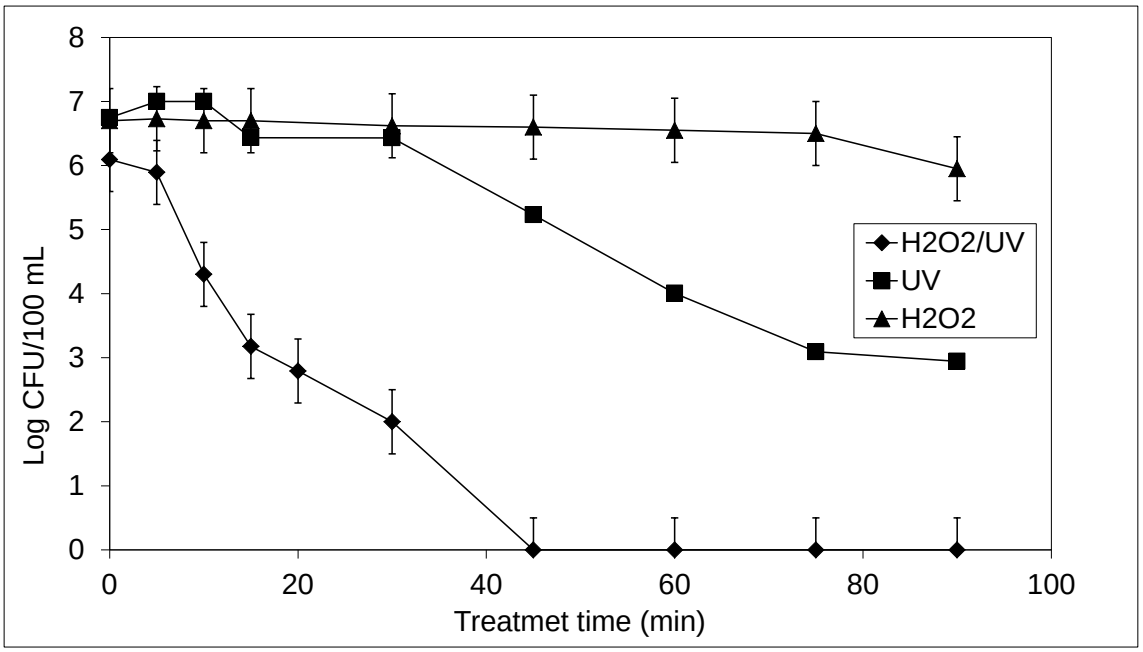

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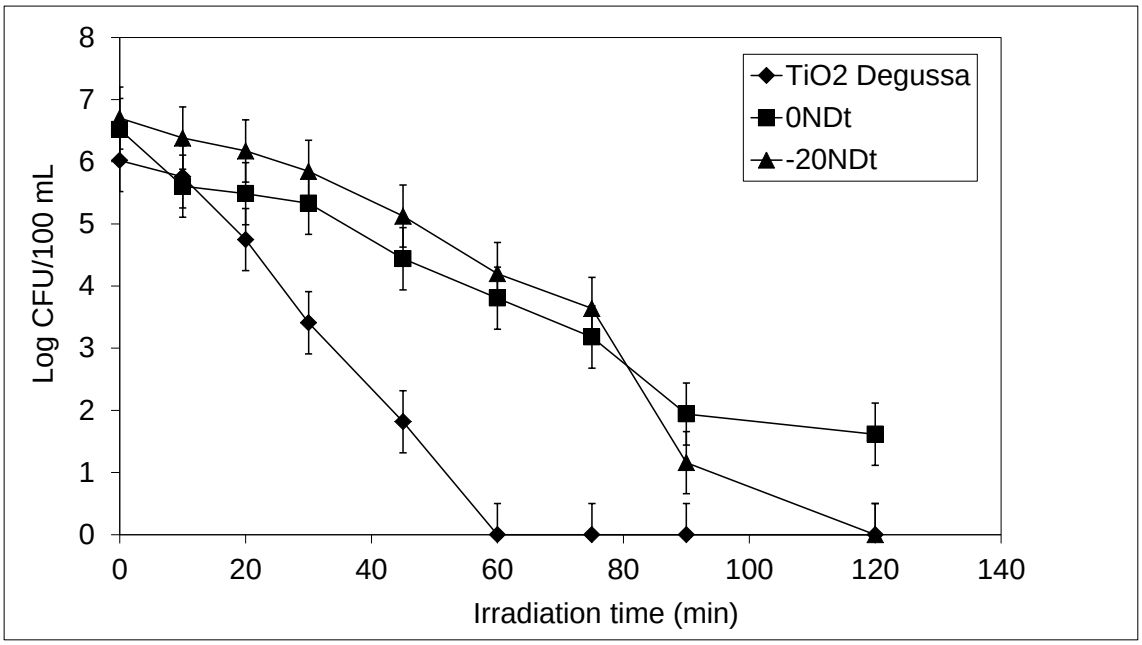

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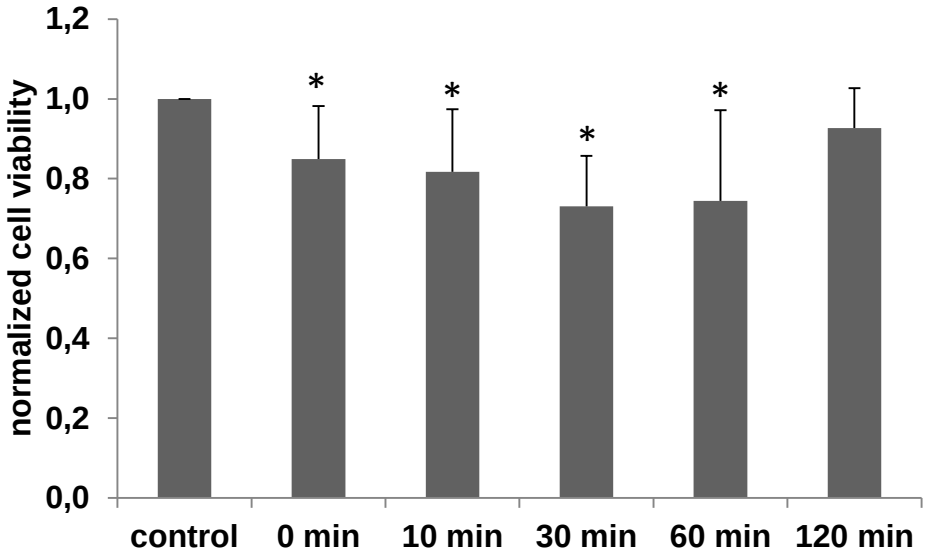

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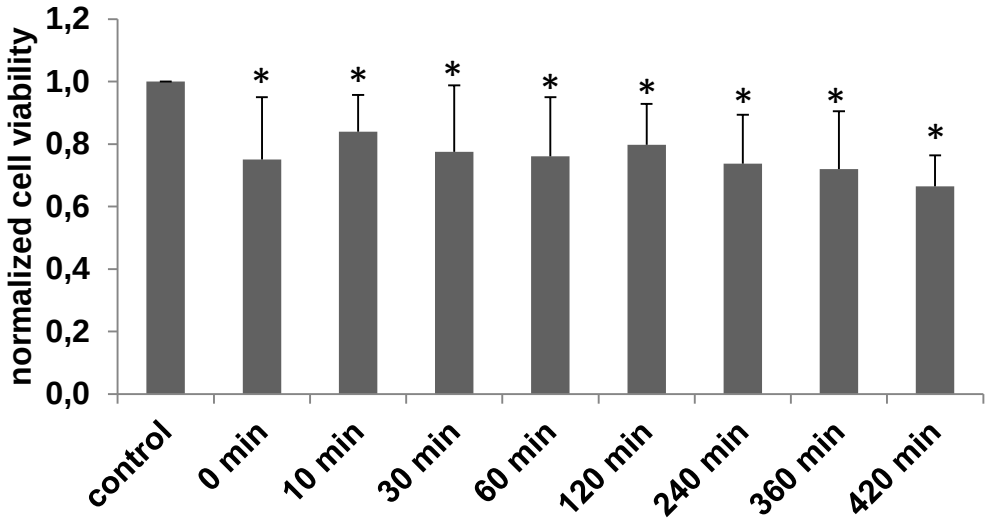

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