Biofilm control in water by a UV-based advanced oxidation process

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An ultraviolet (UV)-based advanced oxidation process (AOP), with hydrogen peroxide and medium-pressure (MP) UV light (H\textsubscript{2}O\textsubscript{2}/UV), was used as a pretreatment strategy for biofilm control in water. Suspended \textit{Pseudomonas aeruginosa} cells were exposed to UV-based AOP treatment, and the adherent biofilm formed by the surviving cells was monitored. Control experiments using H\textsubscript{2}O\textsubscript{2} or MP UV irradiation alone could inhibit biofilm formation for only short periods of time (\textleq 24 h) post-treatment. In a H\textsubscript{2}O\textsubscript{2}/filtered-UV (\textgtrsim 295 nm) system, an additive effect on biofilm control was shown vs filtered-UV irradiation alone, probably due to activity of the added hydroxyl radical (OH\textbullet). In a H\textsubscript{2}O\textsubscript{2}/full-UV (ie full UV spectrum, not filtered) system, this result was not obtained, possibly due to the germicidal UV photons overwhelming the AOP system. Generally, however, H\textsubscript{2}O\textsubscript{2}/UV prevented biofilm formation for longer periods (days) only when maintained with residual H\textsubscript{2}O\textsubscript{2}. The ratio of surviving bacterial concentration post-treatment to residual H\textsubscript{2}O\textsubscript{2} concentration played an important role in biofilm prevention and bacterial regrowth. H\textsubscript{2}O\textsubscript{2} treatments alone resulted in poorer biofilm control compared to UV-based AOP treatments maintained with similar levels of residual H\textsubscript{2}O\textsubscript{2}, indicating a possible advantage of AOP.

\textbf{Keywords:} advanced-oxidation; biofilm; biofouling; disinfection; ultraviolet; water-treatment

\section*{Introduction}

Biofouling is the unwanted deposition of suspended microorganisms on surfaces (Flemming 2002). Biofouling creates a serious operational problem in all water sectors, including piping, water-distribution systems, filtration processes, cooling facilities and power plants (US EPA 2002). Moreover, mature biofilm is persistent and difficult to completely eradicate (Flemming 2002; Kierek-Pearson and Karatan 2005). Common methods for controlling biofouling or regrowth in water include applying disinfectants in the feed water as a pretreatment to limit the growth of suspended cells and further regrowth. Other means of fouling control or removal include, for example, chemical and physical cleaning-in-place (CIP) after the biofilm has formed. In this study, an ultraviolet (UV)-based advanced oxidation process (AOP) was investigated as a preventive strategy for inactivation of suspended cells and consequently, biofilm control.

In AOPs, pollutants are chemically oxidized by free hydroxyl radicals (OH\textbullet) (Singer and Reckhow 1999). OH\textbullet can be generated, for example, by the combined application of ozone/hydrogen peroxide (O\textsubscript{3}/H\textsubscript{2}O\textsubscript{2}), UV radiation/ozone (UV/O\textsubscript{3}), UV/titanium dioxide (UV/TiO\textsubscript{2}) or UV/H\textsubscript{2}O\textsubscript{2}. The most studied AOP is the combination of UV irradiation with the addition of H\textsubscript{2}O\textsubscript{2}. UV irradiation damages DNA by dimerizing adjacent thymine molecules. Formation of a thymine dimer inhibits further transcription of the cell’s genetic code, thereby preventing reproduction of the organism (Setlow and Setlow 1967), while the organism itself is not damaged. However, although the main advantage of UV irradiation is bacterial reduction without the formation of harmful disinfection byproducts (DBPs), it has the disadvantage of not providing a residual bacteriostatic effect. Current technology for water disinfection by UV includes two basic types of mercury lamps, viz. low-pressure (LP) UV mercury vapor lamps that emit a single monochromatic wavelength which peaks at 253.7 nm, and medium-pressure (MP) UV mercury lamps with a broad polychromatic spectrum and output at multiple wavelengths throughout the 200 to 300 nm germicidal UV range and beyond. The addition of H\textsubscript{2}O\textsubscript{2} to the UV process includes two direct reactions (direct photolysis by UV and direct oxidation by H\textsubscript{2}O\textsubscript{2}) and an indirect reaction (oxidation by highly reactive OH\textbullet generated from the direct photolysis of H\textsubscript{2}O\textsubscript{2} by UV light, as shown in Equation (1)).

\begin{equation}
\text{H}_2\text{O}_2 \xrightarrow{\text{hv}} 2 \text{OH}\textbullet
\end{equation}
The \( \text{OH}^\bullet \) is a short-lived, extremely powerful oxidizing agent, able to efficiently oxidize organic molecules. Thus, AOPs have high potential as a substitute for biocide applications (reviewed by Kornmueller 2007). In the inactivation of microorganisms, the efficiency of \( \text{OH}^\bullet \) is generally accepted to be controlled by two main mechanisms, viz. (1) oxidation and disruption of the cell wall and membrane of the microorganism, and (2) diffusion of the \( \text{OH}^\bullet \) into the cell where it may inactivate enzymes, damage intracellular components, and interfere with, eg, protein synthesis and DNA structure.

There are several reports on the disinfecting and oxidizing activity of \( \text{OH}^\bullet \), formed by various methods, on bacteria and viruses (Lubello et al. 2002; Koivunen and Heinonen-Tanski 2005; Alkan et al. 2007; Mamane et al. 2007; Labas et al. 2009). These studies have specifically demonstrated the potential of \( \text{H}_2\text{O}_2/\text{UV} \) as a potential strategy for inactivating microorganisms such as \textit{Escherichia coli}, total coliforms, \textit{Enterococcus faecalis}, \textit{Salmonella enteritidis}, \textit{Bacillus spp.}, and bacteriophages MS2, T4, and T7 (Lubello et al. 2002; Koivunen and Heinonen-Tanski 2005; Alkan et al. 2007; Mamane et al. 2007; Labas et al. 2009).

Kornmueller (2007) presented the advantages of using AOPs, stating that, due to the wide diversity of microorganisms (viruses, bacteria and algae) in water, no single disinfectant is capable of neutralizing all of them at once. Earlier studies showed synergistic effects in the inactivation of suspended vegetative bacteria and bacterial spores by using \( \text{LP UV (254 nm)} \) lamps with the addition of extremely high concentrations of \( \text{H}_2\text{O}_2 \) (ie 10,000 mg l\(^{-1}\)), compared to the use of \( \text{LP UV irradiation, alone} \) (Bayliss and Waites 1979, 1980; Gardner and Shama 1998). Lubello et al. (2002) found synergy between \( \text{H}_2\text{O}_2 \) and \( \text{LP UV irradiation for inactivation of indicator bacteria at \text{H}_2\text{O}_2 \text{ concentrations >20 mg l}^{-1}\text{ in secondary effluent water. Labas et al. (2009) did not observe any additional efficiency in disinfection of \textit{E. coli} when using \text{LP UV with \text{H}_2\text{O}_2 \text{ at 90 to 350 mg l}^{-1}\text{ compared to \text{LP UV alone}}. These authors concluded that addition of \text{H}_2\text{O}_2 \text{ at these concentrations produces a negative effect on the AOP. Other studies investigated the synergistic inactivation of \textit{E. coli} by near-UV (300–420 nm) irradiation with added \text{H}_2\text{O}_2 \text{ (Hartman and Eisenstark 1978). Those authors showed repairable and non-repairable damage to RecA (a DNA repair protein) with the addition of low (0.2–20 mg l}^{-1}\text{ and high (>20 mg l}^{-1}\text{) \text{H}_2\text{O}_2 \text{ concentrations, respectively. Mamane et al. (2007) showed that addition of 25 mg l}^{-1}\text{ \text{H}_2\text{O}_2 \text{ to a filtered \text{MP UV (>295 nm)} irradiation system may result in enhanced disinfection of bacteria and viruses, depending on the type of microorganism evaluated.}

In summary, a few studies have shown the efficiency of \( \text{H}_2\text{O}_2/\text{UV} \) in inactivating different suspended microorganisms. However, there are gaps in the literature with respect to the use of \( \text{OH}^\bullet \), and \( \text{H}_2\text{O}_2/\text{UV} \) in particular, for the prevention of biofilms in water. The treatment with UV-based AOP presented in this study is aimed at controlling or retarding biofilm formation in water; it is not a treatment for the eradication of existing mature biofilms. The influence of \( \text{H}_2\text{O}_2/\text{UV} \) on the inactivation of suspended biofilm-forming bacteria and, consequently, on biofilm control was examined using \( \text{MP UV lamps. The effect of treatment with polychromatic UV-based AOP on the development of biofilms in an aqueous suspension was determined using the model organism \textit{Pseudomonas aeruginosa PAO1 and a model biofilm-formation assay.}

Materials and methods

\textit{Experimental set-up}

Aliquots of 30 ml of \textit{P. aeruginosa PAO1} suspended in phosphate buffer saline (PBS) at an initial concentration of \( \sim 10^6 \text{ colony-forming units (CFU) ml}^{-1}\) were exposed to different treatments (different UV spectra and doses in the absence or presence of \( \text{H}_2\text{O}_2 \) at different concentrations). PBS was used as a controlled aqueous matrix, maintaining constant pH level, without addition of other matrix parameters (eg organic matter and alkalinity). This enabled examination of the different processes in a controlled and simple model system, obtaining reproducible results. After irradiation, each sample was serially diluted, viable cells were enumerated to determine the reduction in suspended cells, and dose–response curves were developed. Subsamples of the treated samples (\( \text{H}_2\text{O}_2 \), UV or \( \text{H}_2\text{O}_2/\text{UV} \)) were further incubated in M9 minimal medium (Sambrook and Russell 2001) at 30°C for short (up to 24 h) and long (several days) incubation periods. Then, biofilm formation was evaluated using high-throughput methods (96 microtiter plate assay) and visualization techniques (confocal laser scanning microscopy, CLSM 510, Zeiss).

‘Full-UV’ experiments included the full spectrum of the MP lamp (200 nm and above). A neutral density (ND) filter (\( \sim 15\% \) transmittance) (type OFR-1.0, POB 82, Caldwell, NJ, USA) was placed in the polychromatic light path to enable appropriate exposure times for acceptable counting ranges of microbial inactivation (without changing the relative polychromatic spectrum). ‘Filtered-UV’ experiments involved the application of a 295-nm long-pass (295-LP) filter (type NT46-418, Edmund Optics, NJ, USA) to filter out the germicidal wavelengths (200–295 nm) and allow wavelengths above \( \sim 295 \text{ nm} \). When combining filtered UV with \( \text{H}_2\text{O}_2 \), the direct UVC...
germicidal photolysis was minimized, and the effect of OH• (over UV photons) in the AOP system could be elucidated.

The main conditions tested were: (1) H₂O₂ alone – effect of H₂O₂ only; (2) MP UV exposure alone – effect of UV photons only; (3) H₂O₂/full-UV – combined effect of UV photons, OH•, and residual H₂O₂; (4) H₂O₂/filtered-UV (>295 nm) – combined effect of filtered UV photons, OH• and residual H₂O₂; (5) H₂O₂/UV/catalase – addition of the antioxidant enzyme catalase (immediately after exposure) to eliminate (by quenching) the residual H₂O₂, leaving the effect of UV photons and OH• only in the H₂O₂/full-UV/catalase system or filtered UV photons and OH• radicals only in the H₂O₂/filtered-UV/catalase system. Figure 1 summarizes all of the conditions tested in the experiment.

**Preparation and enumeration of model bacteria**

*P. aeruginosa* PA01 was used as the model bacterium because it is ubiquitous in water systems and frequently found in biofilms. Inocula of pure cultures were grown in minimal M9 medium and incubated overnight at 30°C with shaking. The inocula were diluted and incubated at 30°C to mid-exponential phase at a concentration of approximately 10⁸ CFU ml⁻¹. Working suspensions for UV exposures were prepared by 100-fold dilution of the 10⁸ CFU ml⁻¹ sample in sterile PBS to a cell count of approximately 10⁶ CFU ml⁻¹. Enumeration was performed by plating on Difco LB agar plates and incubating at 25°C for 2 days.

**UV exposures**

UV exposures were carried out with a MP bench-scale UV collimated-beam apparatus using a 0.45 kW polychromatic lamp (Ace-Hanovia Lamp, Cat. No. 7830-61). The emission spectrum of the UV lamp is shown in Figure 2a. The transmission curves for both ND and 295-LP filters, as illustrated in Figure 2b, were determined with a spectrophotometer (Cary Bio100, Varian, Inc., Palo Alto, CA, USA) equipped with a 150-mm diameter integrated sphere (IS) attachment (Diffuse Reflectance accessory (DRA)-CA-3330, LabSphere, NH, USA). The integrated average irradiance (Eavg) in the samples between 220 and 300 nm, to which the microorganisms were exposed when the filters were used, was calculated according to Bolton and Linden (2003) using the incident spectral irradiance obtained from a calibrated spectroradiometer (USB4000, Ocean Optics, FL, USA, measured without filters), multiplied (weighted) by the transmittance at each wavelength, and taking into account the spectral absorbance of the water (with or without addition of H₂O₂), reflection at the sample surface and the measured Petri-factor for the dish. The UV dose was calculated by multiplying the actual Eavg by exposure time.

Samples were placed in glass dishes, with or without addition of H₂O₂, and irradiated while stirring. Experiments were conducted in sets of 0, 5, and 10 mg l⁻¹ added H₂O₂, corresponding to integrated Eavg values of 0.1356, 0.1354 and 0.1317 mW cm⁻², respectively, with the ND filter, and of 0.0454, 0.0455 and 0.0445 mW cm⁻², respectively, with the 295-LP filter. Additional experiments

![Figure 1. Experimental conditions tested.](image-url)
with 1 and 50 mg l\(^{-1}\) added H\(_2\)O\(_2\) using the 295-LP filter resulted in integrated E\(_{\text{avg}}\) values of 0.0463 and 0.0433 mW cm\(^{-2}\), respectively, and addition of 50 mg l\(^{-1}\) H\(_2\)O\(_2\) with a *1\% transmittance ND filter (type OFR-2.0, POB 82, Caldwell) resulted in an E\(_{\text{avg}}\) value of 0.0100 mW cm\(^{-2}\). Each set was exposed to different exposure times (0, 15, 30, 60 s for the OFR-1.0 ND filter; 0, 300, 600, 900 s for the 295-LP filter; 0, 240, 420, 660, 900 s for the OFR-2.0 ND filter). The mean concentration (CFU ml\(^{-1}\)) of suspended microorganisms spiked in suspension without UV exposure (*10\(^6\) CFU ml\(^{-1}\)) was taken as the initial bacterial concentration, N\(_0\), while the arithmetic mean bacterial concentration per dose was N\(_D\). Regression analysis was performed to fit the linear sections of the log inactivation curve. The dose–response curve was computed as log reduction [\(\log_{10}(N_0/N_D)\)] as a function of UV fluence (dose) with first-order kinetics (\(k = \) inactivation rate coefficient, cm\(^2\) mJ\(^{-1}\)). The kinetic values were calculated using a minimum of four data points, each averaged from at least triplicates.

**Analytical measurements**

Residual H\(_2\)O\(_2\) was measured by the KI-thiosulfate method outlined by Klassen et al. (1994). A stock solution of the antioxidant enzyme catalase (Cat. No. 219001, Calbiochem) was used for detoxification or quenching of residual H\(_2\)O\(_2\). The catalase dry concentration was \(\geq 5000\) units mg\(^{-1}\) dry weight. The final concentration of the catalase stock was 0.1 mg ml\(^{-1}\), i.e. 500 units ml\(^{-1}\). A volume of 50 \(\mu\)l of the catalase stock solution was used for the removal of 10 ppm H\(_2\)O\(_2\) (0.29 mM) from 1 ml of sample.

**Biofilm formation and quantification**

In addition to viable counts, biofilm-formation assays were conducted. Samples before (control) and after H\(_2\)O\(_2\), UV or H\(_2\)O\(_2\)/UV pretreatments were diluted in M9 medium and further incubated at 30°C without shaking for short (<24 h) or long (several days) incubation times. Biofilm formation (as a percentage of the control) was evaluated using a standard method (O’Toole and Kolter 1998), which allows examination of biofilm formation in many experimental conditions in parallel (batch conditions), as detailed in Lakretz et al. (2010). Confocal laser scanning microscopy (CLSM) was used to quantify the effect of the AOP treatment on the viability and adherence of P. aeruginosa PAO1, and on the production of extracellular polymeric substances (EPSs). Biofilm viability was determined using a double live/dead staining kit (BacLight Bacterial Viability Kits, Invitrogen Molecular Probes) containing nucleic acid stains SYTO 9 and propidium iodide (PI). In addition, lectin concanavalin A (Con A)-conjugated Alexa Fluor 647 (Invitrogen Molecular Probes) was used to stain glycoconjugates in the biofilm communities as an indication of EPS (Neu et al. 2001). The samples were prepared by enabling cells to adhere to Thermofax coverslips (Nunc) incubated in M9 medium at 30°C, without shaking. The coverslips were then rinsed, double/triple-stained and visualized by CLSM (Zeiss, LSM 510), with the following excitation/emission detectors and filter sets: for Con A, 650/668, for SYTO 9, 480/500 and for PI, 490/635.

**Results and discussion**

**Effect of treatments on inactivation of suspended cells**

Microorganisms in biofilms differ from their suspended counterparts, thus these two forms were studied separately. The first goal of this research was to evaluate the influence of the H\(_2\)O\(_2\)/UV process and the relative effect of each of the different components (H\(_2\)O\(_2\), UV photons, \(\text{OH}^*\)) on inactivation of
suspended \( P.\ aeruginosa \) PAO1. This goal was addressed by conducting \( \text{H}_2\text{O}_2 \) and UV control tests, and by combining \( \text{H}_2\text{O}_2 \) and UV in the AOP, with or without filtering out the UVC germicidal wavelengths between 200 and 280 nm.

**Effect of \( \text{H}_2\text{O}_2 \)**

There was no bacterial inactivation by 5 or 10 mg l\(^{-1}\) \( \text{H}_2\text{O}_2 \) during 90 min of contact time. These results are in agreement with Bianchini et al. (2002), who demonstrated no coliform inactivation using up to 20 mg l\(^{-1}\) \( \text{H}_2\text{O}_2 \) at a contact time of 30 min, and Sommer et al. (2004), who showed no inactivation of \( \Phi X174 \) phage with 50 mg l\(^{-1}\) \( \text{H}_2\text{O}_2 \) at a contact time of 3 h. Further, Mamane et al. (2007) showed no inactivation of \( E.\ coli, B.\ subtilis \) spores, or MS2, T4 or T7 phages using \( \text{H}_2\text{O}_2 \) at up to 25 mg l\(^{-1}\) and a contact time of 60 min. Labas et al. (2009) concluded that the use of \( \text{H}_2\text{O}_2 \) alone, even at concentrations > 100 mg l\(^{-1}\), is not practical for bacterial inactivation.

**Effect of UV vs \( \text{H}_2\text{O}_2/\text{UV} \)**

The UV spectrum was modified as follows: (1) full-UV experiments, including wavelengths 200–280 nm and above, and (2) filtered-UV experiments (> 295 nm), minimizing the direct UVC photolysis to enable elucidating the effect of \( \text{OH}^* \) (over UV photons). Filtered-UV included wavelengths in the UVB range (≈ 280–315 nm), UVA range (315–400 nm), and visible range (above 400 nm) (see Figure 2b). Two possible mechanisms of microbial inactivation in the UVB wavelength range are known, viz. direct cell death by pyrimidine dimer formation and other lethal photoproducts, and indirect oxidative damage by reactive oxygen species (ROS) mediated, for example, by \( \text{H}_2\text{O}_2 \) or the presence of natural organic matter in the water. In this study, the relative cause of cell death due to direct UVB damage (first mechanism) was determined in a control study (filtered-UV experiments). Additional damage in the \( \text{H}_2\text{O}_2 \)/filtered-UV system, if obtained, could then be related to the \( \text{OH}^* \) activity (second mechanism), due to the presence of \( \text{H}_2\text{O}_2 \).

Figure 3 illustrates the log inactivation of suspended \( P.\ aeruginosa \) PAO1 in buffered water as a function of UV dose using: (1) the full-UV MP spectrum, (2) the filtered-UV (> 295 nm) spectrum, in the presence of 0, 5, 10, and 50 mg l\(^{-1}\) \( \text{H}_2\text{O}_2 \). Dose–response data were fitted using a linear regression approach, to calculate the inactivation rate constant, \( k \). Linear inactivation curves are characteristic of a one hit-one target survival curve (Harm 1980); however, the kinetics of UV inactivation of microorganisms in UV or AOP systems in water is often more complex than simple log-linear inactivation (Mamane 2008). Although curves in this study were not entirely linear across the UV doses tested (a slightly concave curve was obtained), data were still fitted to a linear model. Data were extrapolated through the zero point, and the linear correlation coefficient \( (R^2) \) ranged between 0.93 and 0.99, indicating a good fit of the regression line to the data (Figure 3).

The fluence (dose)-based UV and UV-AOP inactivation rate constant, \( k \), showed values from 0.3594 to 0.4102 cm\(^2\) mJ\(^{-1}\) for full-UV experiments (fluences up to ~ 8 mJ cm\(^{-2}\)), and values from 0.0368 to 0.0773 cm\(^2\) mJ\(^{-1}\) for filtered-UV (> 295 nm) experiments (fluences up to ~ 40 mJ cm\(^{-2}\)). Thus, when filtering the wavelengths between 200 and 295 nm, \( k \) values were ~ 10-fold lower than with the full-UV system, indicating significant enhancement of suspended cell inactivation efficiency for full-UV light compared to filtered-UV light. Indeed, in a previous study (Lakretz et al. 2010), inactivation of suspended \( P.\ aeruginosa \) PAO1 by wavelength bands between 254 and 270 nm (close to the maximum DNA absorbance) was shown to be
most efficient relative to all other evaluated wavelength bands.

Overall, addition of up to 50 mg l\(^{-1}\) H\(_2\)O\(_2\) to the full-UV (MP, up to 8 mJ cm\(^{-2}\)) process did not improve inactivation of suspended \(P.\ aeruginosa\) PAO1 cells compared to full-UV (MP) alone (Figure 3a). Similarly, previous studies have shown no or only mild improvement in the inactivation effectiveness of suspended coliforms using LP UV (120 mJ cm\(^{-2}\)) with ~5 or ~10 mg l\(^{-1}\) added H\(_2\)O\(_2\), compared to LP UV irradiation, alone (Bianchini et al. 2002; Lubello et al. 2002). Lubello et al. (2002) showed synergy between H\(_2\)O\(_2\) and UV at higher added H\(_2\)O\(_2\) (>20 mg l\(^{-1}\)) compared to UV alone. Koivunen and Heinonen-Tanski (2005) concluded that the combination of H\(_2\)O\(_2\)/UV using 3 to 150 mg l\(^{-1}\) H\(_2\)O\(_2\) and a LP-UV dose of 8 to 38 mJ cm\(^{-2}\) does not produce any synergy, and only slightly influences enteric microorganism inactivation compared to UV treatment alone. Labas et al. (2009) showed that AOP applied with 90 to 215 or 150 to 350 mg l\(^{-1}\) H\(_2\)O\(_2\) together with a 15 or 40 W LP-UV lamp, respectively, for inactivation of suspended \(E.\ coli\), had a negative effect on the AOP compared to UV alone. These observations were explained by the high absorbance levels of lethal UVC irradiation by the H\(_2\)O\(_2\) molecules (at these high concentrations) and, also, by the possible formation of protective mechanisms (eg protective shell) in their presence. In general, at high H\(_2\)O\(_2\) concentrations, a competitive reaction for OH\(^*\) becomes significant, and may provide a screening effect that reduces direct photolysis of the model microorganism.

These results suggest an overwhelming effect of the germicidal UVC photons, compared to the OH\(^*\) formed under the above-mentioned AOP conditions. In addition, the mentioned bacteria (ie \(P.\ aeruginosa\), coliforms) are UVC-sensitive, and thus easily affected by UVC photons alone. In natural ecosystems, however, different bacteria and other microorganisms (eg algae) may be involved in biofilm formation and, thus, the addition of H\(_2\)O\(_2\) and the formation of OH\(^*\) in an AOP system may have an advantage over the use of UVC alone, when less UVC-sensitive microorganisms are considered. In practice, UV-based AOP is generally used for oxidation of organic pollutants that require UV doses orders of magnitude higher than those required for disinfection (ie normally in the hundreds to thousands of mJ cm\(^{-2}\)). Kornmueller (2007) suggested that AOPs can be used for disinfection and biofouling control in different marine water applications due to their combination of several oxidants acting simultaneously and, thus, their potential for breaking down different microbial protective mechanisms (eg pigments or strong cell membranes).

To achieve synergy between the AOP components compared to UV irradiation alone (full-UV MP spectrum), several parameters should be taken into consideration: (1) the UV lamp used (LP or MP), (2) the UV dose, (3) the amount of added H\(_2\)O\(_2\) (too low or too high will not be efficient), (4) water type (which may contain UV scavengers or promoters), and (5) the microorganism used. Alkan et al. (2007), for example, showed increased disinfection efficiency of coliforms when adding 3 mg l\(^{-1}\) H\(_2\)O\(_2\) to LP-UV irradiation (68–681 mJ cm\(^{-2}\)) compared to UV alone in humic waters containing fulvic acid (FA), especially at a dissolved organic carbon (DOC) concentration of 10 mg l\(^{-1}\). These authors concluded that a sufficient H\(_2\)O\(_2\) concentration is required to overcome the counteractive effect of FA on UV-disinfection efficiency.

Figure 3b demonstrates log inactivation of suspended \(P.\ aeruginosa\) PAO1 as a function of filtered-UV (>295 nm) fluence (dose). Addition of 10 mg l\(^{-1}\) H\(_2\)O\(_2\) to the filtered-UV system somewhat improved (more significantly at higher doses, ie ~40 compared to 20 mJ cm\(^{-2}\) UV) the efficiency of suspended cell inactivation compared to the 5 mg l\(^{-1}\) H\(_2\)O\(_2\)/filtered-UV system and the filtered-UV system, alone. Moreover, addition of 50 mg l\(^{-1}\) H\(_2\)O\(_2\) to the filtered-UV system further increased suspended cell inactivation compared to additions of 5 or 10 mg l\(^{-1}\) H\(_2\)O\(_2\) or UV irradiation alone.

These results are in accordance with previous results of Hartman and Eisenstark (1978), who showed a synergistic effect of \(E.\ coli\) killing by near-UV (300–420 nm) irradiation with low (ie 0.2–20 mg l\(^{-1}\)) and high (ie >20 mg l\(^{-1}\)) added H\(_2\)O\(_2\), and of Mamane et al. (2007), who showed additional disinfection effect of T7, MS2, and \(E.\ coli\) by adding H\(_2\)O\(_2\) at 25 mg l\(^{-1}\) to a filtered-UV (MP, >295 nm) irradiation system. These authors also observed higher inactivation using the H\(_2\)O\(_2)/UV process for MS2 phage in surface water vs buffered water and explained these results by the plausible formation of ROS other than OH\(^*\), as a result of the presence of organic matter in the water.

The probable synergistic effect in the filtered-UV-AOP system compared to filtered UV alone, could be explained by the contribution of the OH\(^*\) formed in this process, in addition and relative to the UV (long-wave, less energetic) photon activity. The slight overlap between the UV emission spectra using the 295 nm filter and the molar absorption coefficient of H\(_2\)O\(_2\) (together with the compound’s quantum yield) will result in low production of OH\(^*\). The contribution of OH\(^*\) is suggested as a mechanism in the filtered system due to the added effect of increasing H\(_2\)O\(_2\) concentration. In the full-UV-AOP system, the contribution of OH\(^*\) appears to be negligible compared to the lethal UVC (short-wave, highly energetic) wavelengths, and
thus no synergy is seen, at least under the experimental conditions reported here.

**Effect on biofilm control**

The second goal of this research was to determine whether the extent of inactivation of the suspended model microorganism by UV-based AOP is associated with biofilm prevention. To address this issue, potential biofilm formation (as a percentage of the control) by the surviving irradiated bacteria was evaluated. Herein, the term ‘surviving bacterial concentration’ corresponds to log inactivation values and describes the concentration of colony-forming bacteria that survived the pretreatment with UV-based AOP, before the incubation step used to determine biofilm formation (for example, a sample consisting of $10^6$ CFU ml$^{-1}$ bacteria which was treated with UV-based AOP and showed 2-log inactivation corresponds to a surviving bacterial concentration of $10^4$ CFU ml$^{-1}$).

It is important to note that, in real water systems, the first stage of biofilm formation, prior to the attachment of bacteria, corresponds to the adsorption of inorganic molecules or organic macromolecules (eg proteins, carbohydrates, humic acids) to the surface (Compere et al. 2001; Flemming 2002; Bakker et al. 2003). This stage is termed ‘conditioning film’ and does not necessarily depend on the presence of viable microbes. In the current study, this aspect was not investigated. The added M9 minimal medium, after the UV-AOP treatment and prior to the biofilm assay, only enabled bacterial growth and biofilm formation in a controlled model system. Additionally, using PBS as the aqueous matrix for suspending bacterial cells limits the applicability of the conclusions on bacterial inactivation and further biofilm formation to this matrix.

Figure 4 illustrates the percentage biofilm formation obtained after short (<24 h) and long (several days) incubation periods following the different treatments (control, H$_2$O$_2$ alone, UV alone and combinations of H$_2$O$_2$/UV with or without addition of catalase) by the full-UV or filtered-UV (>295 nm) systems in the presence of 10 mg l$^{-1}$ added H$_2$O$_2$. Table 1 shows biofilm formation (% relative to control condition) of *P. aeruginosa* PAO1 after short (<24 h, left) and long (days, right) incubation times following the above treatments in the presence of 1 and 5 mg l$^{-1}$ added H$_2$O$_2$.

**Catalase control tests**

The ability of catalase itself to affect bacterial growth was examined. No effect was found using 50 µl of the catalase stock solution per 1 ml of a control sample. In addition, 5 µl of stock solution was found sufficient for the removal of 10 mg l$^{-1}$ H$_2$O$_2$ from 1 ml of sample during a 24-h control test.

**Effect of H$_2$O$_2$**

Under H$_2$O$_2$ treatment alone, 10 mg l$^{-1}$ H$_2$O$_2$ prevented biofilm formation for short incubation periods post-treatment but not for long incubation periods (Figure 4a and 4b). Treatments with 1 or 5 mg l$^{-1}$ H$_2$O$_2$ alone were insufficient for biofilm prevention, even at short incubation times post-treatment (Table 1). Thus, it could be concluded that H$_2$O$_2$ treatment alone may not be sufficient for biofilm prevention at concentrations that allow regrowth after adequate times post-treatment.

**Effect of UV treatment**

Under UV irradiation alone and the short (<24 h) incubation period post-treatment, the percentage biofilm formation was found to decrease with increasing UV doses in both full-UV and filtered-UV processes (Figure 4a and 4b). At these short incubation times, biofilm formation by *P. aeruginosa* PAO1 was shown to depend mostly on the surviving bacterial concentration after the pretreatment (and prior to the biofilm tests), regardless of the type of UV treatment or photon energy determined by its wavelength (UVC or UVB). For example, ~1.5 log reduction obtained with ~4 mJ cm$^{-2}$ full-UV (corresponding to 30 s) or ~41 mJ cm$^{-2}$ filtered-UV (corresponding to 900 s) resulted in 27% biofilm formation (relative to the control) for both processes (Figure 4a and 4b).

These results are in agreement with a previous study (Lakretz et al. 2010), showing that biofilm control of *P. aeruginosa* PAO1 using the full spectrum of the MP lamp is mostly dependent on UV dose/log inactivation. However, when investigating certain narrow wavelength bands between 239 and 280 nm (filtering out wavelengths below and above the central wavelength), it was shown that biofilm control is most efficient at the central wavelengths of 254–260–270 nm, thus showing a dependence on both surviving bacterial concentration and the targeted UV wavelength used to achieve that particular count. The additional exposure to wavelengths in the visible range (within the polychromatic spectrum of the MP lamp) during exposure to other UV wavelengths, in both full and filtered (>295 nm) UV processes, may play a role in the control of regrowth and biofilm formation, and thus no spectral dependence of biofilm formation was seen. However, this needs to be further investigated.

Looking at the long incubation periods, biofilm formation post-UV treatments reached values similar...
to those in the control non-treated sample (Figure 4b). Overall, for the long incubation periods, UV irradiation alone was not sufficient for biofilm prevention. These results are in agreement with previous studies showing that UV irradiation (mostly by LP lamps) by itself does not have a significant impact on controlling biofilm formation in drinking-water-distribution systems using various methods and systems (Schwartz et al. 2003; Dykstra et al. 2007; Rand et al. 2007; Murphy et al. 2008; Wenjun and Wenjun 2009).

**H₂O₂/UV/catalase vs UV alone**

H₂O₂/UV samples with added catalase post-treatment were compared with those subjected to UV treatment alone (full/filtered), in an attempt to characterize the

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**Figure 4.** Biofilm formation (%) by *P. aeruginosa* PAO1 after short (<24 h, left) and long (days, right) incubation periods post-treatment with (a) full-UV, and (b) filtered-UV (>295 nm) spectra. Treatments (left to right): □ Non-treated control sample (100% biofilm); ■ 10 mg l⁻¹ H₂O₂ treatment alone; □ full-UV(15/30/60 s) or filtered-UV(5/10/15 min) alone; □ 10 mg l⁻¹ H₂O₂/full-UV(15/30/60 s) or 10 mg l⁻¹ H₂O₂/filtered-UV(5/10/15 min) with addition of catalase (no H₂O₂ residual post-treatment); ■ 10 mg l⁻¹ H₂O₂/full-UV(15/30/60 s) or 10 mg l⁻¹ H₂O₂/filtered-UV(5/10/15 min) without addition of catalase (including H₂O₂ residual effect). All samples were incubated in M9 at 30°C without shaking.
additive or synergistic effect of the added OH• and UV photons in preventing biofilm formation. H2O2/UV samples without catalase were compared with the catalase-treated samples, and with the samples treated with H2O2 alone, to explore the role of the residual H2O2 in controlling biofilm formation. The residual H2O2 effect was also investigated in relation to the bacterial concentrations present during the different treatments.

In general, no significant differences were observed in the percentage biofilm formation between full-UV and H2O2/full-UV/catalase treatments, at any of the UV doses tested, regardless of incubation period (<24 h, days), and with both 5 and 10 mg l⁻¹ added H2O2 (Figure 4a and Table 1[a]). These results are in agreement with those found with P. aeruginosa PAO1 after short (<24 h) and long (days) incubation periods post-treatment, using (a) full-UV with 5 mg l⁻¹ H2O2, and filtered-UV (>295 nm) with (b) 5 mg l⁻¹ H2O2 and (c) 1 mg l⁻¹ H2O2.

Table 1. Biofilm formation (%) of P. aeruginosa PAO1 after short (<24 h, left) and long (days, right) incubation periods post-treatment, using (a) full-UV with 5 mg l⁻¹ H2O2, and filtered-UV (>295 nm) with (b) 5 mg l⁻¹ H2O2 and (c) 1 mg l⁻¹ H2O2.

<table>
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<tr>
<td>Control</td>
<td>H2O2</td>
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<td>(1) Control</td>
<td>100 ± 16</td>
<td>100 ± 4</td>
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<tr>
<td>(2) H2O2 5 mg l⁻¹</td>
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<td>84 ± 6</td>
</tr>
<tr>
<td>(3) Full-UV</td>
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<td>40 ± 8</td>
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<tr>
<td>(4) Full-UV + OH• (catalase)</td>
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<tr>
<td>(5) Full-UV + OH• + residual H2O2</td>
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<td>3 ± 1</td>
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<th>9 days</th>
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<tr>
<td>Control</td>
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<tr>
<td>(1) Control</td>
<td>100 ± 19</td>
<td>100 ± 7</td>
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<tr>
<td>(2) H2O2 5 mg l⁻¹</td>
<td>79 ± 5</td>
<td>100*</td>
</tr>
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<td>(3) Filtered-UV</td>
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<td>55 ± 5</td>
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<td>59 ± 11</td>
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<td>(5) Filtered-UV + OH• + residual H2O2</td>
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<td>Control</td>
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<tr>
<td>(1) Control</td>
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<td>100 ± 7</td>
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<tr>
<td>(2) H2O2 1 mg l⁻¹</td>
<td>87 ± 4</td>
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<td>59 ± 11</td>
<td>39 ± 6</td>
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<tr>
<td>(5) Filtered-UV + OH• + residual H2O2</td>
<td>60 ± 5</td>
<td>38 ± 11</td>
</tr>
</tbody>
</table>

Note: Treatments: same as in Figure 4. *Results approximated to 100%.
action, when UVC wavelengths were absent. This effect was not observed in the \( \text{H}_2\text{O}_2/\text{full-UV}/\text{catalase} \) processes (full-UV + \( \text{OH}\bullet \)) compared to full-UV treatments alone, probably due to the lethal effect of the \( \text{UV} \) germicidal photons over the \( \text{OH}\bullet \) contribution (discussed in previous section).

In the long term (ie days), none of the \( \text{H}_2\text{O}_2/\text{UV}/\text{catalase} \) treatments (full/filtered) were sufficient for biofilm prevention (Figure 4, Table 1). These results suggest that those treatments (without residual \( \text{H}_2\text{O}_2 \)) do not provide a sufficient solution for biofilm prevention in the longer term. However, a residual effect may be present in natural water. For example, Lund and Hongve (1994) reported a biofilm-inhibitory effect which remained for at least 1 week in humic surface water that was UV-irradiated. Those authors attributed the effect to the oxidizing species (eg \( \text{OH}\bullet \)) which may have been produced. Alam and Ohgaki (2002) found that UV irradiation had a persistent residual effect in synthetic water in the presence of organic content or transition metals. This effect was extended in the presence of \( \text{H}_2\text{O}_2 \), and was suggested to involve the formation of reactive species (eg \( \text{OH}\bullet \)).

\( \text{H}_2\text{O}_2/\text{UV} \) vs \( \text{H}_2\text{O}_2/\text{UV}/\text{catalase}, \text{UV}, \text{and H}_2\text{O}_2 \)

Most of the \( \text{H}_2\text{O}_2/\text{UV} \) treatments without catalase post-treatment and, thus, with residual \( \text{H}_2\text{O}_2 \) (\( \text{H}_2\text{O}_2/\text{UV} \)), showed a significant biofilm-prevention effect (compared to all \( \text{H}_2\text{O}_2 \) alone, UV alone, and \( \text{H}_2\text{O}_2/\text{UV/catalase treatments} \)). This effect was significant for samples maintained with 10 mg \( \text{l}^{-1} \) added \( \text{H}_2\text{O}_2 \) (all cases) and 5 mg \( \text{l}^{-1} \) added \( \text{H}_2\text{O}_2 \) (depending on UV dose and, thus, on microbial inactivation, at least \( \sim 1-1.5 \) log), but not with 1 mg \( \text{l}^{-1} \) added \( \text{H}_2\text{O}_2 \). This biofilm-prevention effect could be explained by relating the residuals from the added 5 or 10 mg \( \text{l}^{-1} \) \( \text{H}_2\text{O}_2 \) to the surviving bacterial concentration post-treatment. Interestingly, \( \text{H}_2\text{O}_2/\text{UV} \) treatments with residual \( \text{H}_2\text{O}_2 \) from 5 and 10 mg \( \text{l}^{-1} \) showed improved biofilm control compared to 5 and 10 mg \( \text{l}^{-1} \) \( \text{H}_2\text{O}_2 \) treatments alone.

Previous studies have also shown that biofilm control and regrowth prevention can be achieved by an improved synergistic effect between UV irradiation and added disinfectants such as free chlorine, chlorine dioxide and monochloramine (Dykstra et al. 2007; Rand et al. 2007; Murphy et al. 2008; Wenjun and Wenjun 2009). Dykstra et al. (2007) suggested that MP-UV irradiation increases bacterial vulnerability when used before exposure to residual disinfectant in a drinking-water-distribution system. In addition, they suggested that UV pretreatment can reduce disinfectant demand, and thus decrease disinfection by-products (DBPs) formation. Rand et al. (2007) found that, although UV irradiation did not lower disinfectant requirements (dosage), it did reduce by-product formation, at least in the case of UV/\( \text{ClO}_2 \). Momba et al. (1998) observed that the successful combination of an effective primary disinfection process with the presence of effective disinfectant residuals should be taken into account when attempting to limit biofilm formation in water. Those authors found that \( \text{H}_2\text{O}_2 \) at 19.43 mg \( \text{l}^{-1} \) had a longer residual effect on biofilm and regrowth control than chlorine (2.50 mg \( \text{l}^{-1} \)), ozone (2.60 mg \( \text{l}^{-1} \)) or LP UV (30 mW m\( ^{-2} \), 5.4 l min\( ^{-1} \) flow rate), during treatment for 8 days. Alkan et al. (2007) showed complete elimination of surviving bacterial regrowth after \( \text{H}_2\text{O}_2/\text{UV} \) using 0.125 and 3.000 mg \( \text{l}^{-1} \) \( \text{H}_2\text{O}_2 \). They attributed this observation to the residual disinfection effect of the \( \text{H}_2\text{O}_2 \) and the radical species. In addition, they suggested that \( \text{H}_2\text{O}_2 \), although usually requiring long reaction times when used on its own, may be highly beneficial in the AOP system with humic surface waters in increasing the UV disinfection efficiency and preventing bacterial regrowth after exposure. Thus, the above results could be attributed to an additive or synergistic effect of UV photons and the presence of \( \text{H}_2\text{O}_2 \), either by production of the reactive \( \text{OH}\bullet \), or by an efficient combination of primary UV disinfection followed by secondary oxidation by an oxidant that maintains its residual effect (\( \text{H}_2\text{O}_2 \)).

The results suggest a correlation between biofilm control and the concentration of bacteria surviving UV-AOP pretreatment, the UV dose, and the residual \( \text{H}_2\text{O}_2 \) concentration. Moreover, 5 and 10 mg \( \text{l}^{-1} \) \( \text{H}_2\text{O}_2 \) treatments alone resulted in poorer biofilm control compared to AOP pretreatment using full/filtered-UV when similar levels of \( \text{H}_2\text{O}_2 \) residuals were maintained. Thus, further experiments were performed to find a correlation between the role of the ratio of surviving bacterial concentration (CFU \( \text{ml}^{-1} \)) post-AOP treatment to \( \text{H}_2\text{O}_2 \) residual concentration (mg \( \text{l}^{-1} \)) with respect to biofilm control (see next section).

**Ratios of bacterial concentration to \( \text{H}_2\text{O}_2 \) concentration**

Figure 5a illustrates the percentage biofilm formation (relative to the control) of different samples consisting of different ratios of suspended bacterial concentration (achieved by dilution, CFU \( \text{ml}^{-1} \)) to \( \text{H}_2\text{O}_2 \) concentration (mg \( \text{l}^{-1} \)). This experiment was aimed at demonstrating the impact of the ratio of surviving bacterial concentration post-UV-based AOP treatment to the residual \( \text{H}_2\text{O}_2 \) concentration, on biofilm control.

The highest percentage biofilm formation was obtained for the ratio \( 10^5:5 \) of suspended bacterial concentration to \( \text{H}_2\text{O}_2 \) concentration, then for \( 10^6:10 \) at 95 h incubation time. The \( 10^6:5 \) sample showed biofilm formation even at short times (ie < 24 h), whereas the
10^6:10 sample resulted in biofilm formation at longer incubation periods (ie ~ 40 h or more). None of the other samples (10^5:10, 10^5:5, 10^4:10, 10^4:5, 10^2:10, 10^2:5) showed any increase in biofilm formation at any incubation time. These results imply that residual H_2O_2 concentrations of 5 and 10 mg l^{-1} were not sufficient for controlling biofilm formation with a bacterial concentration of 10^6 CFU ml^{-1} suspended *P. aeruginosa* PAO1, whereas these H_2O_2 concentrations were sufficient for controlling biofilm formation with a bacterial concentration of 10^5 CFU ml^{-1} or less.

These results are in agreement with those obtained after H_2O_2/UV (full/filtered) treatments that maintained residual H_2O_2. It is important to note that, in the bench-scale collimated-beam experiments, during the time required for the UV and UV-based AOP experiments (ie seconds to minutes), the change in H_2O_2 initial concentration was not significant, as only a small fraction of the H_2O_2 was consumed in this water and generated OH-. Most of the H_2O_2 was consumed in the time periods after treatment, as can be seen in Figure 5b. Samples maintaining ~10 mg l^{-1} residual H_2O_2 directly after any combination of H_2O_2/UV (full/filtered) resulted in complete long-term prevention of biofilm formed by surviving bacteria at a concentration of up to 6 × 10^5 CFU ml^{-1} (Figure 4a and 4b). In addition, H_2O_2/UV (full/filtered) samples with ~5 mg l^{-1} residual H_2O_2 post-treatment also exhibited complete biofilm prevention, but only for samples with up to 7 × 10^5 CFU ml^{-1} of surviving bacteria, whereas in samples consisting of 4 × 10^5 to 2 × 10^6 CFU ml^{-1} post-H_2O_2/filtered-UV, biofilm control could not be maintained (Table 1[a] and 1[b]).

Figure 5b illustrates the rate of decrease in residual H_2O_2 (ie consumed by the bacteria) for the samples presented in Figure 5a. Time zero relates to the time at which the residual H_2O_2 was analyzed immediately after preparing the suspension of bacterial cells with H_2O_2. Only samples with ratios of 10^6:5 and 10^4:10 suspended bacterial concentration to H_2O_2 concentration consumed all of the H_2O_2 during the first 24 h. All of the other samples consumed H_2O_2 at lower rates, and stabilize at a residual H_2O_2 of 5 to 7.5 mg l^{-1} and 1 to 2.2 mg l^{-1} for samples with initial H_2O_2 concentrations of 10 mg l^{-1} and 5 mg l^{-1}, respectively. Thus, control of bacterial regrowth depends on maintaining a sufficient residual H_2O_2 concentration post-treatment relative to the surviving bacterial concentration immediately after treatment. It appears that samples treated with 5 and 10 mg l^{-1} H_2O_2 alone were not successful at maintaining biofilm prevention due to consumption of all of the residual H_2O_2 by the 10^6 CFU ml^{-1} bacteria during the first 24 h, leaving no H_2O_2 to maintain biofilm control. To conclude, the ratio of the surviving bacterial concentration (after UV-based AOP treatment) to the residual H_2O_2 concentration plays an important role in biofilm prevention.

In the case of applying UV-based AOP to control biofouling in drinking water distribution systems, an additional residual disinfectant (ie chlorine-based) post-AOP treatment should be taken into consideration. The possible expected synergies between all of the process components in such case should be further investigated regarding biofouling control. In addition, chemical selection should be made considering pipe material. Murphy et al. (2008) showed that, when applying ClO_2 or NH_2Cl after UV irradiation in cast iron pipes, corrosion or nitrification could be enhanced and, thus, decrease disinfection efficiency and biofouling control.
**CLSM observation of the adherent biofilm**

The ability of *P. aeruginosa* PAO1 to form an adherent biofilm after UV-based AOP pretreatment, the viability of the formed biofilm cells, and the production of EPS were assessed by CLSM using double live/dead staining with the addition of Con A as a third stain. Figure 6 shows representative CLSM images of biofilms that were grown after short (17 h) and long (10 days) incubation times after control (non-treated, Figure 6a and 6c, respectively) and H$_2$O$_2$/UV treatment with 10 mg l$^{-1}$ added H$_2$O$_2$ (with H$_2$O$_2$ residual, Figure 6b and 6d, respectively). After incubation for 17 h, most of the adhering control (non-treated) bacteria were live (green cells) and evenly dispersed across the slide (Figure 6a), whereas only a few bacteria were attached to the slide after UV-AOP treatment (Figure 6b). After incubation for 10 days, using the triple stain, a ~34-μm thick biofilm was formed in the control non-treated sample consisting of mostly live cells (Figure 6c), whereas almost no cells were attached to the slide with the UV-AOP treated sample (Figure 6d). It appears that UV-AOP-treated cells were able to produce some EPS (purple color) although they did not succeed in forming an adherent biofilm (Figure 6d). These results strengthen the hypothesis that there is an additive or synergistic effect between UV irradiation and H$_2$O$_2$ addition in biofilm prevention.

As discussed earlier, H$_2$O$_2$/UV has a direct antimicrobial effect, but also a direct effect on breaking down various macromolecules (eg proteins, humic substances, and polysaccharides), which form the first required ‘conditioning film’ for the primary microbial layer and subsequent biofilm formation. Song et al. (2004) showed that H$_2$O$_2$/UV pretreatment prior to nanofiltration of groundwater significantly reduced organic fouling, and potentially biofouling, by the transformation of humic/hydrophobic NOM molecules and polysaccharides into less sorbable organic acids. Thus, the combination between UV irradiation, OH$^\bullet$, and residual H$_2$O$_2$ can significantly mitigate membrane fouling. The ability of H$_2$O$_2$/UV to breakdown critical macromolecules originally present in natural waters or secreted by the microorganisms (ie EPS) should be taken into account when evaluating UV-based AOP for biofouling control. This issue requires further investigation.

**Summary and conclusions**

The pretreatment combination of H$_2$O$_2$ and UV (UV-based AOP) has high potential as a biofouling-control strategy. UV irradiation alone can prevent biofilm formation, depending on the UV dose and spectrum, but only for limited periods of time post-treatment due to the absence of a residual effect. Furthermore, H$_2$O$_2$ (up to 10 mg l$^{-1}$) alone was not sufficient in the long term for biofilm control and regrowth prevention in the presence of high bacterial concentrations.

Biofilm control obtained with the full-UV-based AOP system, which included germicidal wavelengths (without residual H$_2$O$_2$), was similar to that obtained with full-UV irradiation alone, for all UV exposure times and incubation periods. In this UV-based AOP system, germicidal UVC wavelengths probably screen the impact of the produced OH$^\bullet$, for both suspended cell inactivation and biofilm control. However, filtering out the germicidal wavelengths (leaving wavelengths above >295 nm), enabled observation of the added impact of OH$^\bullet$ with both suspended cells and biofilms.

Generally, H$_2$O$_2$/UV without residual H$_2$O$_2$ post-treatment prevented biofilm formation for only short periods (<24 h). For longer periods, residual H$_2$O$_2$ was required. H$_2$O$_2$ treatments alone resulted in poorer biofilm control compared to AOP pretreatments with similar levels of residual H$_2$O$_2$. It appears that the ratio of the surviving bacterial concentration post-treatment (after the UV-based AOP treatment) to the residual H$_2$O$_2$ concentration plays an important role in biofilm prevention, and should be taken into consideration, to ensure prevention of bacterial regrowth and biofilm formation post-treatment.

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References


