

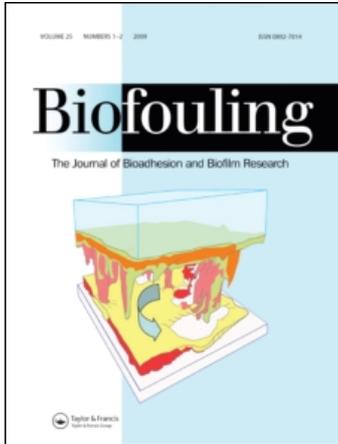
This article was downloaded by: [Tel Aviv University]

On: 10 January 2010

Access details: Access Details: [subscription number 908309149]

Publisher Taylor & Francis

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



## Biofouling

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713454511>

### Biofouling control in water by various UVC wavelengths and doses

Anat Lakretz <sup>ab</sup>; Eliora Z. Ron <sup>b</sup>; Hadas Mamane <sup>a</sup>

<sup>a</sup> School of Mechanical Engineering, Faculty of Engineering, Tel Aviv University, Tel Aviv, Israel <sup>b</sup>

Department of Molecular Microbiology and Biotechnology, Faculty of Life Science, Tel Aviv University, Tel Aviv, Israel

First published on: 18 December 2009

**To cite this Article** Lakretz, Anat, Ron, Eliora Z. and Mamane, Hadas(2010) 'Biofouling control in water by various UVC wavelengths and doses', *Biofouling*, 26: 3, 257 – 267, First published on: 18 December 2009 (iFirst)

**To link to this Article:** DOI: 10.1080/08927010903484154

**URL:** <http://dx.doi.org/10.1080/08927010903484154>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

## Biofouling control in water by various UVC wavelengths and doses

Anat Lakretz<sup>a,b</sup>, Eliora Z. Ron<sup>b</sup> and Hadas Mamane<sup>a\*</sup>

<sup>a</sup>School of Mechanical Engineering, Faculty of Engineering, Tel Aviv University, Tel Aviv 69978, Israel; <sup>b</sup>Department of Molecular Microbiology and Biotechnology, Faculty of Life Science, Tel Aviv University, Tel Aviv 69978, Israel

(Received 28 July 2009; final version received 8 November 2009)

UV light irradiation is being increasingly applied as a primary process for water disinfection, effectively used for inactivation of suspended (planktonic) cells. In this study, the use of UV irradiation was evaluated as a pretreatment strategy to control biofouling. The objective of this research was to elucidate the relative effectiveness of various targeted UV wavelengths and a polychromatic spectrum on bacterial inactivation and biofilm control. In a model system using *Pseudomonas aeruginosa*, the inactivation spectra corresponded to the DNA absorption spectra for all wavelengths between 220 and 280 nm, while wavelengths between 254 nm and 270 nm were the most effective for bacterial inactivation. Similar wavelengths of 254–260–270 nm were also more effective for biofilm control in most cases than targeted 239 and 280 nm. In addition, the prevention of biofilm formation by *P. aeruginosa* with a full polychromatic lamp was UV dose-dependent. It appears that biofilm control is improved when larger UV doses are given, while higher levels of inactivation are obtained when using a full polychromatic MP lamp. However, no significant differences were found between biofilms produced by bacteria that survived UV irradiation and biofilms produced by control bacteria at the same microbial counts. Moreover, the experiments showed that biofilm prevention depends on the post-treatment incubation time and nutrient availability, in addition to targeted wavelengths, UV spectrum and UV dose.

**Keywords:** biofouling; biofilm; ultraviolet; wavelength; water disinfection

### Introduction

Biofouling is the generally accepted term for the unwanted deposition of microorganisms on various surfaces, which in turn interferes with technical, economical, and hygienic requirements (Flemming 2002). Biofouling is a serious problem in all sectors of a water system, including piping, water distribution systems, filtration processes, cooling facilities, and power plants. Biofilms are likely to exist in all water distribution systems (US EPA 2002), as drinking water distribution networks offer very large surface areas for bacteria to attach. In water filtration, biofouling can cause a variety of negative effects on membrane performance, including loss of flux, reduced solute rejection, increased head loss through membrane modules, contamination of permeate, the need for frequent chemical cleanings, degradation of membrane materials, reduced water quality and a reduced membrane life span (Ridgeway and Flemming 1996; Chen et al. 2003; Liao et al. 2004; AWWA 2005). Moreover, the deterioration of water quality due to biofouling and the resultant potential health hazards still remain among the main challenges for water suppliers (Momba et al. 2000).

Chlorination is most frequently applied to drinking water disinfection. However, the risk of elevated levels

of harmful disinfection by-products has given rise to consideration of alternatives for biofouling control in water distribution systems. Oxidative disinfectants may also enhance the formation of easily biodegradable organic substances (BDOC) which can, in turn, be utilized by microorganisms and so promote biofilm formation in water distribution systems (Glaze 1987; Gilbert 1988; Van der Kooij 1999). Moreover, oxidizing biocides used in a pre-treatment step can result in damage to polyamide desalination membranes, thus neutralization of oxidizing agents is required or utilization of an alternative method.

Ultra-violet (UV) irradiation is a non-chemical alternative to control biofouling. UV light irradiation is being increasingly applied as a primary disinfection process for water since it was found to be very effective for inactivating *Cryptosporidium* (Clancy et al. 2000; Craik et al. 2001) and *Giardia lamblia* (Linden et al. 2002) without forming residual disinfection by-products. The mechanism of disinfection by UV light differs from chemical disinfectants such as ozone or chlorine. UV disinfection is effectively used for inactivation of suspended cells, as reviewed by Hijnen et al. (2006). However, microorganisms in biofilms differ from their suspended counterparts regarding their physiology, metabolism, and resistance to

\*Corresponding author. Email: hadasmg@post.tau.ac.il

disinfectants and antibiotics (Engel et al. 1980; Armon et al. 1997; Mackay et al. 1998; Momba et al. 2000; Schwartz et al. 2003; Langmark et al. 2005; Arciola et al. 2008; Moreau-Marquis et al. 2008). Thus, the effectiveness of UV for inactivation of suspended microorganisms may not be relevant when evaluating UV as a strategy to control biofouling.

Current technology for water and wastewater disinfection by UV includes two basic types of mercury lamps: low pressure (LP) UV mercury vapor lamps that emit a single monochromatic wavelength that peaks at 253.7 nm and medium pressure (MP) UV mercury lamps with a broad polychromatic spectrum with output at multiple wavelengths throughout the 220 to 300 nm germicidal UV range and beyond. LP lamps are used for water disinfection due to the high efficiency at the microbiocidal wavelength, while MP lamps have exceptionally high energy output and also prevent the recovery of microorganisms such as *Escherichia coli*, *Cryptosporidium parvum* oocysts, and adenovirus (Chiu et al. 1999; Marshall 1999; Linden et al. 2001; Oguma et al. 2001, 2002; Haider et al. 2002; Kalisvaart 2004; Linden et al. 2007). In addition to DNA and RNA, photochemical reactions may occur also in proteins and enzymes (Harm 1980). The absorbance spectra of proteins show a maximum peak at 280 nm, whereas the peptide bond in proteins displays a significant absorbance below 240 nm due to the large number of these bonds. Therefore, there may be a specific effective UV wavelength for biofouling control, which may suggest an optimization for biofilm control by targeting specific UV wavelengths.

Very few published studies have considered the application of UV irradiation as a potential pre-treatment technology for biofouling control. Most of these studies investigated the impact of monochromatic LP UV light only on biofouling control (except for Munshi et al. 2001 and Dykstra et al. 2007 who studied MP lamps). Several effects of UV disinfection pre-treatment on biofouling control in water distribution systems were demonstrated by Lund and Hongve (1994), Lund and Ormerod (1995), Momba et al. (1998), Schwartz et al. (2003), Pozos et al. (2004), Langmark et al. (2005), Dykstra et al. (2007), Rand et al. (2007), Murphy et al. (2008), and Wenjun and Wenjun (2009). In most cases, UV irradiation by itself did not have a significant impact on controlling biofilm formation in the various models and systems tested. Improved synergetic effects of UV and other disinfectants appear to play an important role in mitigating biofilms and preventing regrowth (Dykstra et al. 2007; Rand et al. 2007; Murphy et al. 2008; Wenjun and Wenjun 2009).

UV radiation also has been evaluated in biofouling prevention for membrane processes such as

microfiltration (MF) (Otaki et al. 1998), nanofiltration (NF) (Munshi et al. 2001), and reverse-osmosis (RO) (Munshi et al. 2001; López-Ramírez et al. 2003). Otaki et al. (1998) found that UV radiation controlled MF membrane fouling by inactivating the microorganisms in the feed and that membrane running time was extended six times longer than the control. The authors concluded that UV did not have a residual effect after irradiation. Munshi et al. (2001) concluded that applying UV ahead of NF membranes influenced biofouling in a seawater (SW) RO desalination plant. These authors suggested the application of UV ahead of NF, and not the RO unit, because low nutrient water can induce starvation and UV resistance.

In the study reported here, the influence of different UVC wavelengths, polychromatic UV light and environmental parameters on the inactivation of biofilm forming bacteria and on biofilm prevention were examined. Thus, the main objectives of the research were to (a) determine the spectral sensitivity of the model organism *Pseudomonas aeruginosa* to wavelengths of 220, 239, 254, 260, 270, 280 nm and the full spectrum using polychromatic MP light; (b) elucidate the relative effectiveness of the various UV wavelengths and full MP UV light on biofilm formation, and (c) determine the impact of nutrients and post-treatment incubation time on biofilm formation.

## Materials and methods

### Experimental design

Water samples spiked with the biofilm forming model bacterium *P. aeruginosa* PAO1 were exposed to UV using a bench scale UV apparatus. These water samples were further used to examine the biofilm formation potential using high throughput methods which could screen many conditions in parallel.

In short, the experimental procedure included: (1) preparing cultures of *P. aeruginosa*; (2) preparing bacterial suspension ( $10^6$  CFU ml<sup>-1</sup>) in phosphate buffer (PBS); (3) exposing microorganisms to UV pretreatment at the desired fluence (dose) and wavelength; (4) determining the viability by colony counts before and after UV; (5) determining the dose-response of *P. aeruginosa* in terms of survival of suspended cells; (6) biofilm formation and quantification by microtitre plate and gentian violet assays; and (7) plotting biofilm formation (%) as a function of survival and UV fluence.

### Preparation and enumeration of the bacterium

*P. aeruginosa* PAO1 (Stover et al. 2000) was used as the model Gram-negative bacterium because it is ubiquitous in water systems and frequently found in biofilms. It has become the most accepted model

organism for studying biofilm formation (Costerton et al. 1987; O'Toole et al. 2000; Stoodley et al. 2002; Klausen et al. 2003). Inocula of pure cultures were grown in minimal M9 medium and incubated overnight at 30°C with shaking. The medium was supplemented with 0.4% glucose as a carbon source. The inocula were diluted and incubated until a cell density of  $\sim 10^8$  colony-forming units per ml (CFU ml<sup>-1</sup>) was obtained. Working suspensions for UV exposures were prepared by 100-fold dilution of *P. aeruginosa* (at  $\sim 10^8$  CFU ml<sup>-1</sup>) in phosphate buffer (PBS) to achieve a cell count of  $\sim 10^6$  CFU ml<sup>-1</sup>. Enumeration was performed on Luria-Bertani (LB) agar plates incubated at 25°C for 2 days.

### Genomic DNA purification

DNA purification from *P. aeruginosa* PAO1 and *E. coli* K12 was carried out using standard techniques (Sambrook et al. 1989). In short, a pellet from overnight cultures was resuspended in lysis buffer, incubated, and treated with biophenol:chlorophorm: isoamyl alcohol to start DNA extraction as described by Sambrook et al. (1989).

### Experimental setup for UV exposures

UV exposures were carried out using a medium pressure (MP) bench scale UV collimated beam apparatus. The UV radiation was directed through a circular opening (collimated tube) to provide incident radiation normal to the surface of the water test suspension. The experiments were carried out using a 0.45 kW polychromatic lamp (Ace-Hanovia Lamp Cat. No. 7830-61) with output at multiple wavelengths throughout the 220–300 nm UV range and beyond. Emission spectrum of the UV lamp is shown in Figure 1a.

Aliquots of *P. aeruginosa* in PBS at initial concentrations of  $\sim 10^6$  CFU ml<sup>-1</sup> were exposed to a range of UV fluence, and dose–response curves were developed. Water samples were placed in glass dishes and irradiated while stirring the samples slowly to avoid vortex on a magnetic stir plate. Each sample was serially diluted, and enumerated to determine the bacterial number by viable cell counts. After irradiation, biofilm formation potential and quantification assays were conducted. Mean concentration (CFU ml<sup>-1</sup>) of microorganisms spiked in suspension without UV exposure was taken as the initial concentration,  $N_0$ , while the arithmetic mean concentration per fluence is  $N_D$ . The log<sub>10</sub> transformation for  $N_0/N_D$  was plotted as a function of the average UV fluence. Regression analysis was performed on all the data fields used to fit the linear sections of the log inactivation curve.

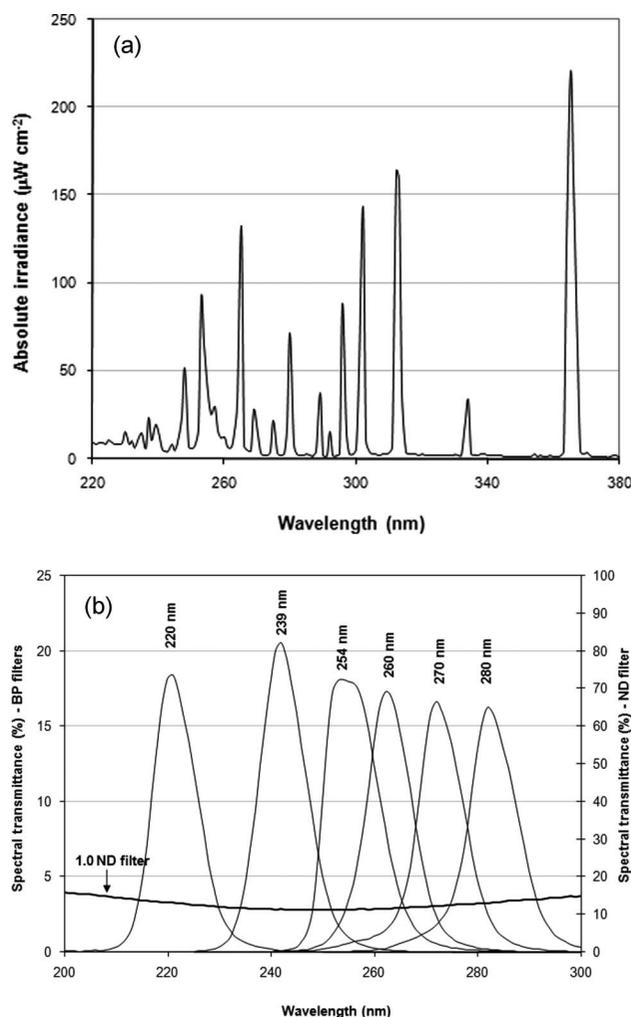


Figure 1. (a) Emission spectrum of full UV MP lamp. (b) Transmission curves of the 220, 239, 254, 260, 270, and 280 nm BP filters (left y axis) together with the transmission curve of the 1.0 ND filter (FDU 1.0) (right y axis).

The integrated average irradiance between 200 and 300 nm was calculated according to Bolton and Linden (2003), using the spectral incident irradiance obtained from a calibrated spectroradiometer (USB4000, Ocean Optics) placed in the same  $x$ ,  $y$  position as the center of the crystallization dish and at the surface of the liquid suspension, the water spectral absorbance obtained *via* a UV–Vis spectrophotometer (Cary Bio100, Varian, Inc., Palo Alto, CA, USA), the reflection at the sample surface and the measured Petri-factor for the dish. The UV fluence was calculated by multiplying the average irradiance with exposure time.

In several experiments, a Neutral Density (ND) filter (type OFR-1.0, POB 82, Caldwell, NJ, USA) was placed in the polychromatic light path to reduce the measured incident irradiance (without changing the relative polychromatic spectrum) at about 85% (corresponding to 15% transmittance) to enable

appropriate exposure times for an acceptable counting range of microbial inactivation that will produce a fluence response curve with sufficient data points. In addition, band-pass (BP) filters placed in the polychromatic light path were used to transmit a well-defined band of light from the polychromatic MP light source at a centre wavelength of 220, 239, 254, 270 and 280 nm with an average full width at half maximum (FWHM) of 20–27.5 nm and minimum peak transmittance ranges between 12 and 15% (corresponds to the ND filter's transmittance range) (Andover Corporation, NH, USA). The transmission curves for the ND and BP filters, as illustrated in Figure 1b, were performed with the same spectrophotometer for absorbance measurement, equipped with a 150 mm diameter IS attachment (Diffuse Reflectance accessory (DRA)-CA-3330, Labsphere, NH, USA). The filter was placed in a holder at the sample transmission port of the integrating sphere. The actual average irradiance, to which the microorganisms were exposed when the filters were used, was obtained by multiplying (weighting) the spectral incident irradiance (measured without filters) by the bandwidth at each wavelength (spectral transmittance in percentage according to Figure 1b), taking into consideration the water spectral absorbance, petri factor and the water reflection as previously described.

#### Determination of biofilm formation potential

Biofilm formation assays were performed by a standard method, as previously described (O'Toole and Kotler 1998; Houdt et al. 2004; Ramsey and Whiteley 2004; Peeters et al. 2008). The ability of bacteria to form biofilms is assessed by the ability of cells to attach to round-bottomed 96-well microtitre plates made of polystyrene (TC Microwell 96U, NUNC). Samples before and after UV irradiation were centrifuged and then diluted in the same volume with M9 or LB nutrient medium. A volume of 200  $\mu$ l of each sample was applied per well in 3–6 replicates, and the plates were further incubated in 30°C (M9) or 37°C (LB) without shaking for 10–37 h.

#### Biofilm quantification – Gentian Violet assay (GV assay)

All samples (with or without UV) were applied to the same microtitre plates, incubated and treated in the same way. After a pre-defined incubation time, the supernatant was gently removed and the wells were rinsed with PBS and allowed to dry on a blotting paper, leaving only adherent bacteria in the wells. The biofilms were fixed by addition of 99% ethanol and air-drying. The plates were then stained with 0.4%

Gentian Violet (GV) solution. The unbounded GV was removed by rinsing the plates under running tap water. The GV-stained biofilm was solubilized with 1% SDS and incubated for 15 min, to enable elution of all the biofilm-associated dye. Finally, 100  $\mu$ l from each well were transferred to a new polystyrene plate, and the absorbance was determined with a plate reader at 590 nm. The effect of UV irradiation was calculated relative to the mean average of the untreated sample that contained  $\sim 10^6$  CFU ml<sup>-1</sup> and constituted the 100% value.

## Results and discussion

### Activity spectrum of *P. aeruginosa*

The main goal of this research was to explore whether the activity spectrum of a suspended model microorganism can effectively predict biofouling prevention as determined by the biofilm formation measurements. To address this issue, the action spectrum of *P. aeruginosa* was investigated. The inactivation of disperse bacterial cells in completely mixed batch reactor is obtained using Equation (1), where the dose–response curve is computed as log reduction ( $\log_{10} N_0/N_D$ ) as a function of UV fluence (dose) and shows first order kinetics:

$$\log\left(\frac{N_0}{N_D}\right) = kI_{\text{avg}}t = kD \quad (1)$$

where  $N_D$  = total number of surviving bacteria at UV dose  $D$ ;  $N_0$  = total number of bacterial cells before UV application (at time  $t = 0$ );  $k$  = inactivation rate (or sensitivity) coefficient, cm<sup>2</sup> mJ<sup>-1</sup>;  $I_{\text{avg}}$  = average irradiance of UV light in bulk solution, mW cm<sup>-2</sup>;  $t$  = exposure time in seconds (s) and  $D$  = UV dose or fluence, mJ cm<sup>-2</sup>.

The log survival of suspended *P. aeruginosa* in buffered water as a function of UV fluence for BP wavelengths of 220, 239, 254, 260, 270, 280 nm, and for the 1.0 ND-filtered full MP lamp is illustrated in Figure 2. Without the ND filter, total inactivation of *P. aeruginosa* occurred after UV exposure for < 1 min; thus, the ND filter was used to produce a fluence–response curve with sufficient data points.

The fluence–response data were fitted using a linear regression approach resulting in inactivation rate constants  $k(\lambda)$ . These constants, also termed UV sensitivity coefficients, are a measure of the sensitivity of a microorganism at a particular wavelength and, when plotted as a function of wavelength, represent the action spectrum of a microorganism (Rauth 1965; Munakata et al. 1986; Cabaj et al. 2001, 2002; Mamane-Gravetz et al. 2005). The kinetics values were calculated using a minimum of four data points

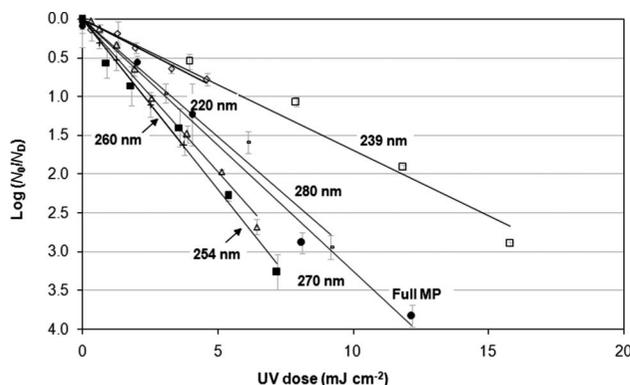


Figure 2. Dose response of *P. aeruginosa* PAO1. Log inactivation as a function of UV filtered wavelengths:  $\diamond$ , 220 nm BP;  $\square$ , 239 nm BP;  $\blacksquare$ , 254 nm BP;  $+$ , 260 nm BP;  $\triangle$ , 270 nm BP;  $-$ , 280 nm BP;  $\bullet$ , Full MP (filtered with 1.0 ND filter).

(per wavelength), each averaged from triplicates (at least). Linear inactivation curves are characteristic of a one hit-one target survival curve that assumes that a single harmful event (hit) is sufficient to inactivate a biological unit (Harm 1980). However, the kinetics of UV inactivation of microorganisms in water is often more complex than a simple log-linear inactivation (Mamane 2008). Although curves in this study were not entirely linear throughout the UV doses tested (a slightly concave curve was obtained), data were fitted to a linear model. Data were extrapolated through the zero point and the linear correlation coefficient ( $R^2$ ) ranged from 0.96 to 1.00, indicating a strong fit of the regression line to the data. The fluence-based UV sensitivity coefficients,  $k(\lambda)$  revealed values from 0.17 to  $0.44 \text{ cm}^2 \text{ mJ}^{-1}$ . Figure 3 illustrates the spectral UV sensitivity coefficient  $k_{\text{rel}}(\lambda)$  for *P. aeruginosa* plotted as a function of UV wavelength and compared with its  $\text{DNA}_{\text{rel}}$  absorbance and with the  $\text{DNA}_{\text{rel}}$  absorbance of *E. coli*, all normalized relative to 254 nm. The action spectrum is typically reported relative to 254 nm, the principal output of the low pressure Hg vapor lamp. Thus the  $k(\lambda)$  from linear regression is transformed to  $k_{\text{rel}}(\lambda)$ , the relative coefficient, by dividing  $k(\lambda)$  by  $k(254)$  (obtained using the 254 nm BP filter) which is  $0.44 \text{ cm}^2 \text{ mJ}^{-1}$ . In addition,  $\text{DNA}_{\text{rel}}$  is the DNA absorbance relative to DNA absorbance at 254 nm, which is 0.77 for *E. coli* and 0.78 for *P. aeruginosa*. The actual values determined for  $k_{\text{rel}}(\lambda)$  can be obtained from data in Figure 3.

Overall, the *P. aeruginosa* action spectra followed the shape of the DNA spectra for all of the wavelengths between 220 and 280 nm. Some wavelengths were more effective than others, in the order  $254 \approx 260 > 270 > \text{Full MP (1.0 ND)} > 280 > 220 > 239 \text{ nm}$ . The wavelengths between 254 nm and

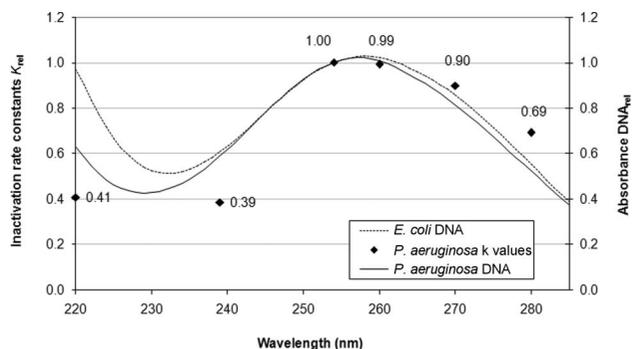


Figure 3. Action spectrum of *P. aeruginosa* PAO1 in comparison to its DNA absorbance and to the DNA absorbance of *E. coli* (all values are normalized to 254 nm).

270 nm were the most effective in the inactivation of suspended *P. aeruginosa* in comparison to ranges above and below it. Similar results were obtained by Linden et al. (2001) regarding inactivation of *C. parvum* in similar UV spectra bands and UV fluences. Gates (1928) discovered that the relative biological effect of various UV wavelengths on *E. coli* matched the absorbance of the cell's DNA. Similarity to the absorbance spectra of DNA (*E. coli* or *P. aeruginosa*) suggests that UV inactivation mechanism of suspended *P. aeruginosa* PAO1 is similar to that of other microorganisms, and is presumably DNA absorbance-dependent, thus wavelength-dependent.

#### Effect of UV wavelengths on biofilm production

The aim of this study was to explore whether there is a specific effective set of UV wavelengths that can be targeted for biofilm control. The relative effectiveness of each wavelength in preventing biofilm formation was examined using the microtitre plate assay and quantified using the GV technique (detailed in Materials and methods). However, it is important to note that this assay is a two-step procedure (i.e. irradiation and then separate biofilm formation from the microbial survivors) and does not mimic natural or engineering processes when surface colonization events and irradiation would be concurrent.

Figure 4 illustrates biofilm formation (%) as a function of log inactivation at different wavelength bands (of 239, 254–270, 280 nm using band-pass filters), and full MP UV light (in this case not filtered with ND filter). Exposure of *P. aeruginosa* to increasing UV doses in each wavelength resulted in increasing log inactivation values in the suspended phase. Afterwards, the irradiated samples were incubated for 10 h in LB medium at  $37^\circ\text{C}$ , and the biofilm formed was quantified. Variation in biofilm formation (%) at each wavelength corresponds to the biofilm formed after

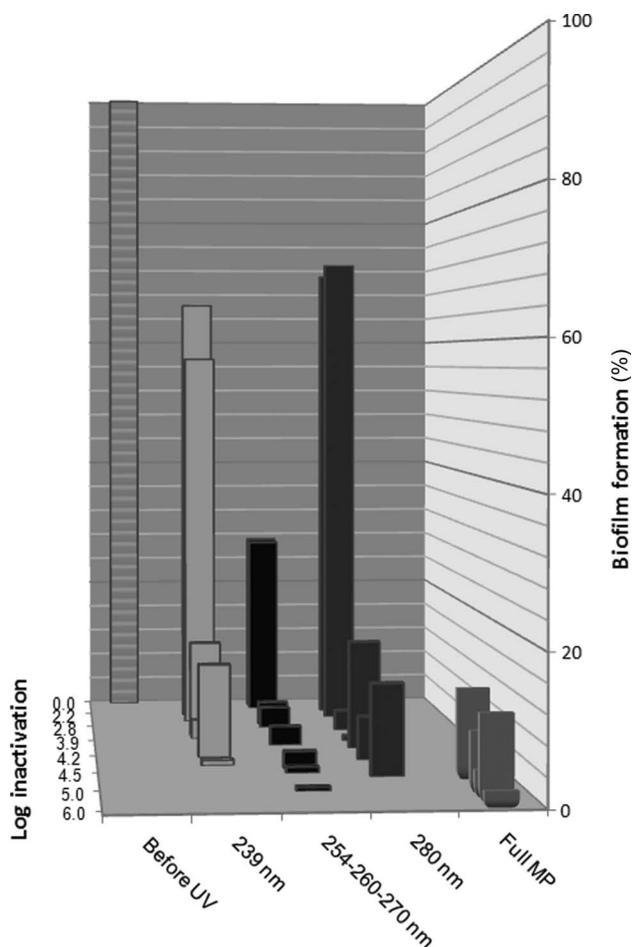


Figure 4. Biofilm formation (%) as a function of log inactivation at different wavelength bands (239, 254–270, 280 nm using BP filters), and full MP UV light (not filtered). Experimental conditions: 10 h incubation, LB medium, 37°C.

different log inactivation values. The results for wavelengths of 254, 260, and 270 nm are presented together, as they are similar. The biofilm formation percentage was calculated compared to a  $10^6$  CFU  $\text{ml}^{-1}$  control sample of a non-UV-irradiated sample which represents the column with 100% value (see methods). The percentage biofilm reduction can be calculated as 100% minus the percentage biofilm formation (Z axis of Figure 4), which is a measure of process efficacy. The term ‘microbial counts’ correspond to log inactivation values and describes the concentration of bacterial cells after UV pretreatment and before incubation for biofilm quantification assay. For example, a control sample consisting of  $10^6$  CFU  $\text{ml}^{-1}$  bacteria that was irradiated and resulted in 2 log inactivation corresponds to a microbial count of  $10^4$  CFU  $\text{ml}^{-1}$ .

Two hypothetical outcomes are plausible: (a) the percentage biofilm reduction depends solely on the

microbial count after pretreatment and prior to biofilm tests, thus biofilm formation should be similar for the same log inactivation regardless of the type of UV pretreatment (various wavelengths and exposure times used to reach a certain log inactivation), and (b) the percentage biofilm formation does not only depend on the microbial count but also on the UV wavelength used to achieve that certain count due to different UV damage at different wavelengths that may impact biofilm formation.

As illustrated in Figure 4, differences in biofilm prevention between 239, 254–270, and 280 nm ranges were observed mostly at log inactivation smaller than 3 log, but also at higher values. At smaller log inactivation values – biofilm formation (%) after 254-260-270 nm treatments were not >30%, while after 239 nm and 280 nm treatments were ~60% and ~70%, respectively. At higher log inactivation values biofilm formation (%) after 254-260-270 nm treatments were not >3%, while after 239 nm and 280 nm treatments were up to 14% and 16%, respectively. Overall, the results indicate that pretreatment with UV wavelengths of 254-260-270 nm were more efficient in biofilm prevention of *P. aeruginosa* compared to wavelengths of 239 and 280 nm. Thus, at similar log inactivation (below 3-log), some wavelengths are more efficient than others and the second hypothesis was confirmed. The trend of these results is also similar to the UV inactivation of suspended *E. coli* (see Figure 3). Consequently, when targeting specific wavelengths, biofilm reduction does not only depend on microbial count, but also on the targeted wavelengths. This may be attributed to UV-induced damage at certain wavelengths of 254, 260 and 270 nm that reduced the ability of the cells to form biofilms under these specific test conditions.

This outcome may also indirectly imply that biofouling prevention using UV irradiation is most effective when DNA is damaged, rather than proteins or peptide bonds that show a maximum peak at 280 nm and 240 nm, respectively (Harm 1980).

Figure 5 illustrates biofilm formation (%) as a function of UV wavelengths at similar UV filtered doses. Results were obtained from incubation for 10 h following UV treatments using LB medium at 37°C. From this figure it is apparent that, for the same UV dose (although different log inactivation), wavelengths of 254-260-270 nm were more effective than 239 and 280 nm and also required less exposure times for bacterial inactivation and biofilm control. As previously discussed (and illustrated in Figure 4), for the same log inactivation values (although different UV doses), wavelengths of 254-260-270 were also more efficient compared to 239 and 280 nm in most cases.

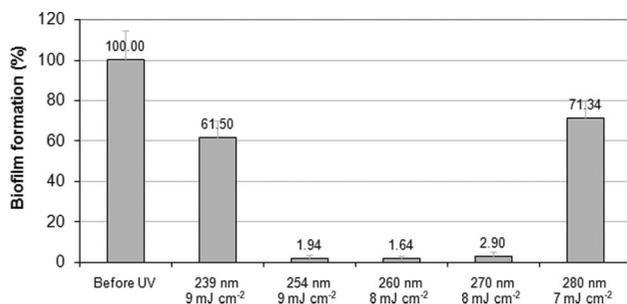


Figure 5. Biofilm formation (%) as function of UV wavelength bands at similar UV doses. Experimental conditions: 10 h incubation, LB medium, 37°C.

### Effect of full spectrum MP-UV light on biofilm formation

Although targeting specific wavelengths may be beneficial, it is clearly not practical to target specific UV wavelengths for water disinfection using MP polychromatic lamps and band-pass filters. Possibly, in the future, UV Light Emitting Diodes (LEDs) can be used to disinfect water at the DNA absorbance peak wavelength range. In water treatment plants, LP lamps are generally used due to the high efficiency at the microbicidal wavelength without producing unwanted photochemical changes in other water constituents (Chiu et al. 1999; Haider et al. 2002). Medium pressure (MP) UV mercury lamps, however, emit a broad polychromatic spectrum with output at multiple wavelengths throughout the 220 to 300 nm germicidal UV range (Figure 1a) and at the visible range, which might be advantageous. Studies have shown that, in order to ensure permanent inactivation and prevent the recovery of microorganisms following UV exposures, MP UV light is required and preferred (Linden et al. 2001, 2007; Oguma et al. 2001, 2002; Kalisvaart, 2004). These wavelength spectra inflict irreversible damage on cellular DNA as well as on other molecules, such as: proteins, enzymes, coenzymes, hormones and electron carriers (Harm 1980). In addition, MP lamps can be advantageous over LP lamps because they have exceptionally high energy output and therefore require less space and fewer lamps in water treatment plants (Marshall 1999).

As illustrated in Figure 4, using full MP UV light (not filtered with a ND filter) gave high log inactivation values even at short exposure times; thus biofilm potential in these set of experiments for full lamp was tested for only above 4 logs of inactivation. To overcome this issue, a ND filter was placed in front of the light source. Figure 6 illustrates biofilm formation after UV irradiation using a full spectrum of MP UV light (filtered with a 1.0 ND filter) at different UV doses, thus different log inactivation

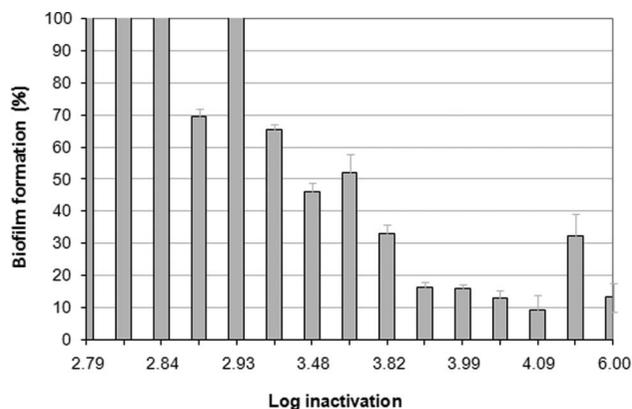


Figure 6. Biofilm formation (%) as a function of inactivation of suspended cells using Full MP light filtered with 1.0 ND filter (~15% transmittance). Experimental conditions: 12 h in LB medium at 37°C.

values. Again, biofilm formation was calculated compared to a control sample of a  $10^6$  CFU ml<sup>-1</sup> non-irradiated sample which comprised the 100% value, and biofilm prevention was considered as 100% minus biofilm formation. All the results in Figure 6 were obtained after 12 h incubation following UV treatments in LB medium at 37°C (10 or 12 h were chosen due to practical experimental reasons). The results indicate, except for deviation in one data point at 4.51 log, that prevention of *P. aeruginosa* biofilm with a full polychromatic lamp is presumably dependent on the inactivation of suspended cells, which is UV dose-dependent. It appears that biofilm control improves when greater UV doses are given, and greater levels of inactivation of suspended cells are obtained.

The last observation has led to a further examination of the possible differences in biofilm formation by irradiated bacteria (ie cells that survived after UV pretreatment filtered with a 1.0 ND filter) and control bacteria (not irradiated) at the same bacterial concentrations (microbial counts). The goal was to examine whether the cells which survived irradiation experienced additional damage that would prevent them from producing biofilm. Figure 7 illustrates biofilm formation (%) as a function of bacterial concentration:  $\sim 10^3$ ,  $\sim 10^2$ , and  $\sim 10^1$  CFU ml<sup>-1</sup> (irradiated and control). The UV-irradiated samples ( $\sim 10^3$ ,  $\sim 10^2$ ,  $\sim 10^1$  CFU ml<sup>-1</sup> correspond to log inactivation values of  $\sim 3$ ,  $\sim 4$ , and  $\sim 5$  log, respectively) were compared to control non-irradiated diluted samples ( $\sim 10^3$ ,  $\sim 10^2$ ,  $\sim 10^1$  CFU ml<sup>-1</sup>) of the same microbial counts. The control samples were obtained by dilution of the original non-irradiated  $\sim 10^6$  CFU ml<sup>-1</sup> sample that comprised the 100% condition. All samples were incubated in M9 minimal medium at 30°C for 20 h. These conditions were

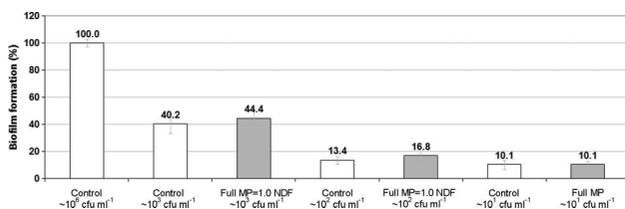


Figure 7. Biofilm formation (%) as a function of bacterial concentration of suspended *P. aeruginosa*. Microbial counts were obtained after UV irradiation (filtered with 1.0 ND filter) or after dilution of the original  $10^6$  CFU ml<sup>-1</sup> control sample. Experimental conditions: 20 h incubation in M9 minimal medium at 30°C.

chosen from practical considerations of having sufficient time between the UV irradiation experiment and the biofilm quantification assay.

No significant differences were found between biofilm formation by irradiated bacteria and biofilm formation by control bacteria at the same microbial counts. Irradiated bacteria at concentrations of  $\sim 10^3$ ,  $\sim 10^2$ ,  $\sim 10^1$  CFU ml<sup>-1</sup> showed 44.4%, 16.8%, 10.1% biofilm formation, respectively. Non-irradiated bacteria at the same concentrations showed 40.2%, 13.4%, 10.1% biofilm formation, respectively. These results strengthen the plausible conclusion that prevention of biofilm formation by *P. aeruginosa* using polychromatic UV pretreatment is mostly bacterial concentration dependent, as it depends on inactivation of suspended cells resulting from the UV dose given.

MP UV light might have advantages over targeting with monochromatic or LP (254 nm) UV lamps, due to its irreversible damage and additional or synergistic effects on microbial inactivation (Marshall 1999; Linden et al. 2001, 2007; Oguma et al. 2001, 2002). Because there is evidence for this damage in suspended cells, it was hypothesized that this is a possibility also for biofilms. However, it has not been proven whether a combined polychromatic MP lamp that provides a full spectrum is more advantageous compared to the wavelengths of 254-260-270 nm. Future studies are required to compare the impact of LP to MP lamps, using various test conditions and long incubation times post treatment, to truly investigate the impact of re-growth.

### Effect of other parameters on biofilm control

#### Time dose reciprocity

With inactivation of suspended microorganisms by UV, it has been previously shown that the same product of intensity-time (time dose reciprocity) may not lead to the same reduction of microorganisms, at different intensities (Sommer et al. 1996). This effect was due to cellular repair enzymes that were more influenced by high UV intensities (Sommer et al. 1998).

Regarding biofilms, Patil et al. (2007) found that the combination of intensity and exposure time is important in biofouling control, and stronger effects on biofouling were observed at higher UV intensities. These authors demonstrated UVC radiation as a potential tool for biofouling control in marine optical instruments and concluded that biofouling prevention is a function of UV intensity, exposure time, water turbidity and the distance between the lamp and coupons (attachment surface). They also concluded that the UV dose was not the sole determinant of the UV effectiveness, and introduced interesting concepts that impact biofilm formation, such as the threshold levels for UV intensity and the duration and mode of UV exposure (continuous or in cycles). It was suggested that there might be an intensity and duration threshold that should be defined regarding UV treatment concerning biofilm control.

Biofilm formation using a non-filtered MP light reached approximately 5–15% at log inactivation above 4.86 (Figure 4). The results for the ND-filtered MP light were 10–15% at log inactivation above 3.94 (Figure 6). Non-filtered MP light showed incident irradiance of  $1.26$  mW cm<sup>-2</sup> (between 200 and 300 nm), while filtered MP light (using a 1.0 ND filter that transmits  $\sim 15\%$  of the irradiance) showed incident irradiance of  $0.15$  mW cm<sup>-2</sup>. Shorter UV exposure times are needed using full MP light compared to filtered MP light in order to obtain the same log inactivation. At the same range of log inactivation values, non-filtered MP light was more effective in biofouling control compared to ND-filtered light due to its high intensity and the short irradiation times, but the differences were small and may be negligible. Although the impact of the time-dose reciprocity data is not available for biofilms, it is presented here as a possibility because there is evidence for time-dose reciprocity in certain suspended cells with UV bench-scale studies. Further work is required using different ND filters (resulting in different UV irradiance values) to examine the impact of time-dose reciprocity on suspended cells and, further, on biofilm formation. It is important to note, however, that the UV intensities in actual applications of UV flow-through reactors is much higher than in bench scale studies, with shorter exposure times (in seconds), which raises the question of the relevance of this theory/observation for UV reactors.

#### Incubation time

Figure 8 illustrates the percentage biofilm formation as a function of log inactivation and of incubation times in M9 minimal medium, following pretreatment using full MP UV light (not filtered). It can be seen that up to

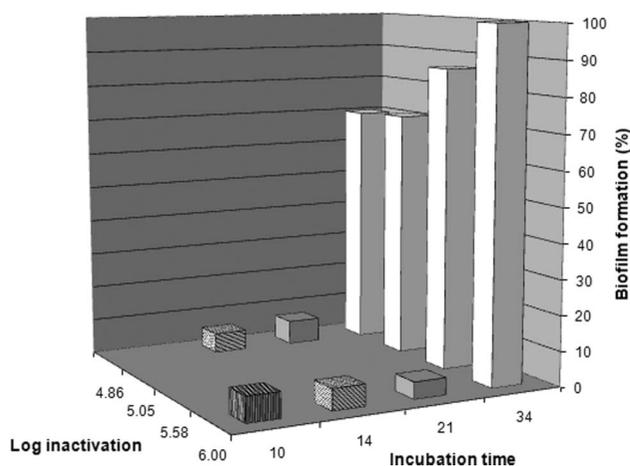


Figure 8. Biofilm formation (%) as a function of inactivation of suspended cells and of different incubation times following full MP UV light pretreatment (not filtered). All samples were incubated in minimal M9 medium at 30°C.

incubation for 21 h in minimal medium post-treatment, biofilm formation was controlled to a level of <10%. It also can be seen that after 34 h incubation, biofilm formation was no longer controlled, and achieved almost the 100% value of a non-irradiated sample. These results may lead to the conclusion that, while applying UV pretreatment for biofouling control, in addition to optimization of UV wavelengths and doses, there is also a need to optimize and determine the mode of UV operation (eg continuous or in cycles) and to define a threshold of maximal incubation time possible post-treatment in the environmental conditions given. This aspect was also discussed by Patil et al. (2007).

#### Environmental conditions (nutrients, temperature)

The effects of UV exposure are also influenced by the environmental conditions during- and post-treatment. Therefore, the influence of M9 minimal medium at 30°C on the biofilm formation by *P. aeruginosa* was determined, compared to rich LB medium at 37°C, following full MP UV pretreatment.

When using full MP UV light filtered with a 1.0 ND filter, it was observed (data are not shown) that biofilm formation post-treatment in LB medium was much faster than that in M9 medium. In LB medium, biofilm formation was controlled only until ~12 h post-treatment, while in M9 medium it was controlled for ~21 h and longer. In addition, biofilm formation in LB medium seemed to be less reproducible and more vulnerable to intrusive procedures such as quantification by the Gentian Violet assay. When using non-filtered MP UV light, it was observed (partially shown in Figure 8) that biofilm formation in both LB medium

and M9 medium was controlled to a level of ~40% and ~10%, respectively, until at least 21 h post-treatment. In summary, it seems that biofilm formation is strongly media-dependent and most probably temperature-dependent as well. These parameters should be taken into consideration while designing UV pretreatment system for biofouling control. In addition, there is a need to distinguish between biofouling prevention, which is pretreatment reducing the concentration of suspended cells in order to prevent biofouling formation, and biofouling treatment, which is treatment against already existing biofilms. Moreover, in certain applications, such as drinking water distribution systems, a combination of UV with other disinfectants should be considered, in order to ensure a residual effect post-treatment.

#### Summary, conclusions, and future suggestions

The *P. aeruginosa* action spectra followed the shape of the DNA spectra for all the wavelength range between 220 and 280 nm, while wavelengths between 254 nm and 270 nm were the most effective in inactivation, suggesting that UV inactivation mechanism is DNA absorbance-dependent. Wavelengths of 254-260-270 nm were more effective than those at 239 and 280 nm and also required shorter exposure times for bacterial inactivation and, in most cases, also for biofilm control. In addition, biofilm prevention by *P. aeruginosa* with a full polychromatic lamp was UV dose-dependent. It appears that biofilm control improves when greater UV doses are given, and greater levels of inactivation of suspended cells are obtained. Moreover, no significant differences were found between biofilm formation by surviving UV-irradiated bacteria and by control bacteria at the same microbial counts.

There is a need for a further investigation in order to determine and optimize all the parameters influencing biofouling control while using UV as a pretreatment strategy. The parameters suggested are: (1) UV design parameters, viz. wavelengths, doses, intensities and duration threshold levels, and mode of UV exposure (for example-continuous or in cycles); (2) environmental parameters, viz. incubation time post-treatment, nutrient availability during- and post-treatment, temperature, microbial composition, and the water matrix. In addition, there is a need to distinguish between a biofouling prevention strategy and existing biofouling treatment. In certain applications, such as drinking water distribution systems, a combination of UV with other disinfectants should be considered, in order to assure a residual effect post-treatment. Validation and verification of this process might lead to step-change advances in water treatment

by means of a pilot scale operating system that will make the newly developed technology prepared for future commercialization.

### Acknowledgements

This research is sponsored by the MAGNET Program, in the Office of the Chief Scientist of the Ministry of Industry, Trade and Labor, Grant number 2668. The authors acknowledge Atlantium Ltd and especially Mr Arnon Meir, Dr Vladimir Glukhman and Dr Tali Harif for their fruitful collaboration and insights into UV, Mr Chen Katz for his assistance with the microbiological methods, and Dr Amir Zlotkin and Mr Hen Kestenboim for their assistance with the GV assay.

### References

- Arciola R, Baldassarri L, Campoccia D, Creti R, Pirini V, Huebner J, Montanaro L. 2008. Strong biofilm production, antibiotic multi-resistance and high gelE expression in epidemic clones of *Enterococcus faecalis* from orthopaedic implant infections. *Biomaterials* 29:580–586.
- Arnon R, Starosvetzky J, Arbel T. 1997. Survival of *Legionella pneumophila* and *Salmonella typhimurium* in biofilm systems. *Water Sci Technol* 35:293–300.
- AWWA Membrane Technology Research Committee. 2005. Committee report: recent advances and research needs in membrane fouling. *J Am Water Works Assoc* 97:79–89.
- Bolton JR, Linden KG. 2003. Standardization of methods for fluence (UV dose) determination in bench-scale UV experiments. *J Environ Eng ASCE* 129:209–215.
- Cabaj A, Sommer R, Pribil W, Haider T. 2001. What means “Dose” in UV disinfection with medium pressure lamps? *Ozone Sci Eng* 23:239–244.
- Cabaj A, Sommer R, Pribil W, Haider T. 2002. The spectral sensitivity of microorganisms used in biosimetry. *Water Sci Technol: Water Supp* 2:175–181.
- Chen CL, Liu WT, Chong ML, Wong MT, Ong ST, Seah H, Ng WJ. 2003. Community structure of microbial biofilms associated with membrane-based water purification processes as revealed using a polyphasic approach. *Appl Microbiol Biotechnol* 70:553–568.
- Chiu K, Lyn DA, Savoye P, Blatchley ER. 1999. Integrated UV disinfection model based on particle tracking. *J Environ Eng* 125:7–16.
- Clancy JL, Bukhari Z, Hargy TM, Bolton JR, Dussert BW, Marshall MM. 2000. Using UV to inactivate *Cryptosporidium*. *J Am Water Works Assoc* 92:97–104.
- Costerton JW, Cheng K-J, Geesey GG, Ladd TI, Nickel JC, Dasgupta M, Marrie TJ. 1987. Bacterial biofilms in nature and disease. *Annu Rev Microbiol* 41:435–464.
- Craik SA, Weldon DA, Finch GR, Bolton JR, Belosevic M. 2001. Interactivation of *Cryptosporidium parvum* oocysts using medium- and low-pressure ultraviolet radiation. *Water Res* 35:1387–1398.
- Dykstra TS, O’Leary KC, Chauret C, Andrews RC, Gagnon GA. 2007. Impact of UV and secondary disinfection on microbial control in a model distribution system. *J Environ Eng Sci* 6:147–155.
- Engel HWB, Berwald LG, Havelaar AH. 1980. The occurrence of *Mycobacterium kansasii* in tapwater. *Tubercle* 61:21–26.
- Flemming HC. 2002. Biofouling in water systems – cases, causes and countermeasures. *Appl Microbiol Biotechnol* 59:629–640.
- Gates FL. 1928. A study of the bactericidal action of ultraviolet light. *J Contam Hydrol* 13:231–260.
- Gilbert E. 1988. Biodegradability of ozonation products as a function of COD and DOC elimination by the example of humic acid. *Water Res* 92:123–126.
- Glaze WH. 1987. Drinking-water treatment with ozone. *Environ Sci Technol* 21:224–230.
- Haider T, Sommer R, Knasmüller S, Eckl P, Pribil W, Cabaj A, Kundi M. 2002. Genotoxic response of Austrian groundwater samples treated under standardized UV (254 nm) disinfection conditions in a combination of three different bioassays. *Water Res* 36:25–32.
- Harm W. 1980. Biological effects of ultraviolet radiation. Cambridge, UK: Cambridge University Press.
- Hijnen WAM, Beerendonk EF, Medema GJ. 2006. Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: a review. *Water Res* 40:3–22.
- Houdt RV, Aertsen A, Jansen A, Quintana AL, Michiels CW. 2004. Biofilm formation and cell-to-cell signalling in Gram-negative bacteria isolated from a food processing environment. *J Appl Microbiol* 96:177–184.
- Kalisvaart BF. 2004. Re-use of wastewater: preventing the recovery of pathogens by using medium-pressure UV lamp technology. *Water Sci Technol* 50:337–344.
- Klausen M, Heydorn A, Ragas P, Lambertsen L, Aes-Jørgensen A, Molin S, Tolker-Nielsen T. 2003. Biofilm formation by *Pseudomonas aeruginosa* wild type, flagella and type IV pili mutants. *Mol Microbiol* 48:1511–1524.
- Langmark J, Storey MV, Ashbolt NJ, Stenström TA. 2005. Accumulation and fate of microorganisms and microspheres in biofilms formed in a pilot-scale water distribution system. *Appl Environ Microbiol* 71:706–712.
- Liao BQ, Bagley DM, Kraemer HE, Lepparad GG, Liss SN. 2004. A review of biofouling and its control in membrane separation bioreactors. *Water Environ Res* 76:425–436.
- Linden KG, Shin G, Sobsey MD. 2001. Comparative effectiveness of UV wavelengths for the inactivation of *Cryptosporidium parvum* oocysts in water. *Water Sci Technol* 43:171–174.
- Linden KG, Thurston J, Schaefer R, Malley JR, Jr. 2007. Enhanced UV inactivation of adenoviruses under polychromatic UV Lamps. *Appl Environ Microbiol* 73:7571–7574.
- Linden KG, Shin GA, Faubert G, Cairns W, Sobsey MD. 2002. UV disinfection of *Giardia lamblia* in water. *Environ Sci Technol* 36:2519–2522.
- López-Ramírez JA, Sahuquillo S, Sales D, Quiroga JM. 2003. Pre-treatment optimisation studies for secondary effluent reclamation with reverse osmosis. *Water Res* 37:1177–1184.
- Lund V, Hongve D. 1994. Ultraviolet irradiated water containing humic substances inhibits bacterial metabolism. *Water Res* 28:1111–1116.
- Lund V, Ormerod K. 1995. The influence of disinfection processes on biofilm formation in water distribution systems. *Water Res* 29:1013–1021.
- Mackay WG, Gribbon LT, Barer MR, Reid DC. 1998. Biofilms in drinking water systems – a possible reservoir for *Helicobacter pylori*. *Water Sci Technol* 38:181–185.
- Mamane H. 2008. Impact of particles on UV disinfection of water and wastewater effluents: a review. *Rev Chem Eng* 24:67–157.
- Mamane-Gravetz H, Linden KG, Cabaj A, Sommer R. 2005. Spectral sensitivity of *Bacillus subtilis* spores and MS2 coliphage for validation testing of ultraviolet reactors for water disinfection. *Environ Sci Technol* 39:7845–7852.

- Marshall T. 1999. Deadly pulses. *Water Environ Technol* 11:37–41.
- Momba MNB, Kfir R, Venter SN. 2000. An overview of biofilm formation in distribution systems and its impact on the deterioration of water quality. *Water SA* 26:59–66.
- Momba MNB, Cloete TE, Venter SN, Kfir R. 1998. Evaluation of the impact of disinfection processes on the formation of biofilms in potable surface water distribution systems. *Water Sci Technol* 38:283–289.
- Moreau-Marquis S, Stanton BA, O'Toole GA. 2008. *Pseudomonas aeruginosa* biofilm formation in the cystic fibrosis airway. *Pulm Pharmacol Ther* 21:595–599.
- Munakata N, Hieda K, Kobayashi K, Ito A, Ito T. 1986. Action spectra in ultraviolet wavelengths (150–250 nm) for inactivation and mutagenesis of *Bacillus subtilis* spores obtained with synchrotron radiation. *Photochem Photobiol* 44:385–390.
- Munshi HA, Saeed MO, Green TN, Al-Hamza AA, Farooque MA, Ismail ARA. 2001. Impact of UV irradiation on controlling biofouling problems in NF-SWRO desalination process. International Desalination Association (IDA) World Congress, Singapore 2005, SO05-170.
- Murphy HM, Payne SJ, Gagnon GA. 2008. Sequential UV- and chlorine-based disinfection to mitigate *Escherichia coli* in drinking water biofilms. *Water Res* 42:2083–2092.
- O'Toole GA, Kotler R. 1998. Initiation of biofilm formation in *Pseudomonas fluorescens* WCS365 proceeds via multiple, convergent signalling pathways: a genetic analysis. *Mol Microbiol* 28:449–461.
- O'Toole GA, Kaplan HB, Kolter R. 2000. Biofilm formation as microbial development. *Ann Rev Microbiol* 54:49–79.
- Oguma K, Katayama H, Ohgaki S. 2002. Photoreactivation of *Escherichia coli* after low- or medium-pressure UV disinfection determined by an endonuclease sensitive site assay. *Appl Environ Microbiol* 68:6029–6035.
- Oguma K, Katayama H, Mitani H, Morita S, Hirata T, Ohgaki S. 2001. Determination of pyrimidine dimers in *Escherichia coli* and *Cryptosporidium parvum* during UV light inactivation, photoreactivation, and dark repair. *Appl Environ Microbiol* 67:4630–4637.
- Otaki M, Takizawa S, Ohgaki S. 1998. Control and modeling of membrane fouling due to microorganism growth by UV pretreatment. *Water Sci Technol* 38:405–412.
- Patil JS, Kimoto H, Kimoto T, Saino T. 2007. Ultraviolet radiation (UV-C): a potential tool for the control of biofouling on marine optical instruments. *Biofouling* 23:215–230.
- Peeters E, Nelis HJ, Coenye T. 2008. Comparison of multiple methods for quantification of microbial biofilms grown in microtiter plates. *J Microbiol Methods* 72:157–165.
- Pozos N, Scow K, Wuertz S, Darby J. 2004. UV disinfection in a model distribution system: biofilm growth and microbial community. *Water Res* 38:3083–3091.
- Ramsey MM, Whiteley M. 2004. *Pseudomonas aeruginosa* attachment and biofilm development in dynamic environments. *Mol Microbiol* 53:1075–1087.
- Rand JL, Hofmann R, Alam MZB, Chauret C, Cantwell R, Andrews RC, Gaynon GA. 2007. A field study evaluation for mitigating biofouling with chlorine dioxide or chlorine integrated with UV disinfection. *Water Res* 41:1939–1948.
- Rauth AM. 1965. The physical state of viral nucleic acid and the sensitivity of viruses to ultraviolet light. *Biophys J* 5:257–273.
- Ridgeway HF, Flemming HC. 1996. Membrane biofouling. In: Maillevalle J, Odendaal PE, Wiesner MR, editors. *Water treatment membrane processes*. New York: McGraw-Hill. p. 6.1–6.6.
- Sambrook J, Fritsch E, Maniatis T. 1989. *Molecular cloning: a laboratory manual*, 2nd ed. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press.
- Schwartz T, Hoffmann S, Obst U. 2003. Formation of natural biofilms during chlorine dioxide and u.v. disinfection in a public drinking water distribution system. *J Appl Microbiol* 95:591–601.
- Sommer R, Haider T, Cabaj A, Heidenreich E, Kundi M. 1996. Increased inactivation of *Sacharomyces cerevisiae* by protraction of UV irradiation. *Appl Environ Microbiol* 62:1977–1983.
- Sommer R, Haider T, Cabaj A, Pribal W, Lhotsky M. 1998. Time dose reciprocity in UV disinfection of water. *Water Sci Technol* 38:145–150.
- Stoodley P, Sauer K, Davies DG, Costerton JW. 2002. Biofilms as complex differentiated communities. *Ann Rev Microbiol* 56:187–209.
- Stover CK, Pham XQ, Erwin AL, Mizoguchi SD, Warrener P, Hickey MJ, Brinkman FSL, Hufnagle WO, Kowalik DJ, Lagrou M, et al. 2000. Complete genome sequence of *Pseudomonas aeruginosa* PA01, an opportunistic pathogen. *Nature* 406:959–964.
- USEPA. 2002. Health risks from microbial growth and biofilms in drinking water distribution systems [Internet]. Office of Water (4601M), Office of Ground Water & Drinking Water; [cited 2009 Dec 1]. Available from [http://www.epa.gov/safewater/disinfection/tcr/pdfs/white\\_paper\\_tcr\\_biofilms.pdf](http://www.epa.gov/safewater/disinfection/tcr/pdfs/white_paper_tcr_biofilms.pdf).
- Van Der Kooij D. 1999. Potential for biofilm development in drinking water distribution systems. *J Appl Microbiol Symp Supp* 85:39S–44S.
- Wenjun S, Wenjun L. 2009. Impact of the UV disinfection process on biofilm control in a model drinking water distribution system. *Environ Eng Sci* 26:809–816.