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Review

Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: A review

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ABSTRACT

UV disinfection technology is of growing interest in the water industry since it was demonstrated that UV radiation is very effective against (oo)cysts of *Cryptosporidium* and *Giardia*, two pathogenic micro-organisms of major importance for the safety of drinking water. Quantitative Microbial Risk Assessment, the new concept for microbial safety of drinking water and wastewater, requires quantitative data of the inactivation or removal of pathogenic micro-organisms by water treatment processes. The objective of this study was to review the literature on UV disinfection and extract quantitative information about the relation between the inactivation of micro-organisms and the applied UV fluence. The quality of the available studies was evaluated and only high-quality studies were incorporated in the analysis of the inactivation kinetics.

The results show that UV is effective against all waterborne pathogens. The inactivation of micro-organisms by UV could be described with first-order kinetics using fluence-inactivation data from laboratory studies in collimated beam tests. No inactivation at low fluences (offset) and/or no further increase of inactivation at higher fluences (tailing) was observed for some micro-organisms. Where observed, these were included in the description of the inactivation kinetics, even though the cause of tailing is still a matter of debate. The parameters that were used to describe inactivation are the inactivation rate constant k (cm^2/mJ), the maximum inactivation demonstrated and (only for bacterial spores and *Acanthamoeba*) the offset value. These parameters were the basis for the calculation of the microbial inactivation credit (MIC = "log-credits") that can be assigned to a certain UV fluence. The most UV-resistant organisms are viruses, specifically Adeno-viruses, and bacterial spores. The protozoon *Acanthamoeba* is also highly UV resistant. Bacteria and (oo)cysts of *Cryptosporidium* and *Giardia* are more susceptible with a fluence requirement of $<20\,mJ/cm^2$ for an MIC of 3 log.

Several studies have reported an increased UV resistance of environmental bacteria and bacterial spores, compared to lab-grown strains. This means that higher UV fluences are required to obtain the same level of inactivation. Hence, for bacteria and spores, a correction factor of 2 and 4 was included in the MIC calculation, respectively, whereas some wastewater studies suggest that a correction of a factor of 7 is needed under these conditions. For phages and viruses this phenomenon appears to be of little significance and for protozoan (oo)cysts this aspect needs further investigation. Correction of the required fluence for DNA repair is considered unnecessary under the conditions of drinking water practice (no photo-repair, dark repair insignificant, esp. at higher ($60\,mJ/cm^2$) fluences) and

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probably also wastewater practice (photo-repair limited by light absorption). To enable accurate assessment of the effective fluence in continuous flow UV systems in water treatment practice, biodosimetry is still essential, although the use of computational fluid dynamics (CFD) improves the description of reactor hydraulics and fluence distribution. For UV systems that are primarily dedicated to inactivate the more sensitive pathogens (*Cryptosporidium, Giardia*, pathogenic bacteria), additional model organisms are needed to serve as biodosimeter.

Contents

1. Introduction

The first application of UV irradiation in drinking water as disinfection process was in 1910 in Marseille (Henry et al., 1910), after the development of the mercury vapour lamp and the quartz tube and establishing the germicidal effect of UV irradiation. According to Wolfe (1990) and Hoyer (2004), general application was hampered because of high costs, poor equipment reliability, maintenance problems and the advent of chlorination (cheaper, more reliable and potential to measure disinfectant residual). Due to the increased information on the production of hazardous oxidation by-products during chlorination and ozonation, UV irradiation gained more attention; low-pressure UV produces almost no by-products. Also, unlike chemical disinfectants, the biological stability of the water is not affected by low-pressure lamps. In Europe, UV has been widely applied for drinking water disinfection since the 1980s, for the control of incidental contamination of vulnerable groundwater and for reduction of Heterotrophic Plate Counts (Kruithof et al., 1992). The breakthrough of UV applicability as a primary disinfection process in the US and Europe came after the discovery of the high efficacy of UV irradiation against *Cryptosporidium* (Clancy et al., 1998) and *Giardia*. Chemical disinfection with chlorine is not effective against these pathogens and ozone applied at low CT values to limit formation of bromate has relatively little effect on the infectivity of the protozoan (oo)cysts. In contrast, infectivity of these pathogens is significantly reduced by UV fluences that can readily be applied in drinking water treatment. UV is now regarded as being broadly effective against all pathogens, bacteria, protozoa and viruses that can be transmitted through drinking water.

The introduction of the quantitative microbiological risk assessment (QMRA) to define the microbiological safety of drinking water (Haas, 1983; Regli et al., 1991; Teunis et al., 1997; Medema et al., 2003) is a development of growing interest. Besides knowledge about the presence of pathogenic

micro-organisms in the source water, QMRA requires quantitative knowledge about the capacity of water treatment processes, including UV disinfection, to eliminate (remove or inactivate) pathogenic micro-organisms.

The aim of this study was to evaluate available literature data and create a well-defined database which enables calculation of the microbial inactivation credit (MIC) of UV disinfection for viruses, bacteria and protozoan (oo)cysts in water. Most studies are lab-scale based. For full-scale chemical disinfection processes with chlorine and ozone, it was demonstrated that the MIC for *Escherichia coli* was lower than expected from the applied CT values and the known dose–response curves determined under laboratory conditions (Hijnen et al., 2000, 2004a). Therefore, literature was evaluated to verify the influence of process conditions on the MIC of full-scale UV disinfection processes. The results of this review were also used to identify further research needs.

2. Materials and methods

2.1. Selection of the reviewed literature

Literature on the inactivation of viruses, bacteriophages, bacteria, bacterial spores and protozoan (oo)cysts by UV irradiation in water was collected and evaluated on technical and microbiological aspects. In the review, only those studies were used where inactivation was assessed using generally accepted microbial culturing (solid media or tissues) or animal infectivity methods. Similarly, only studies were evaluated in which assessment of the UV fluence was clearly described and based on either UV sensor measurements, fluence calculations and/or reduction equivalent fluence (REF) assessment with (bio)dosimetry. The description and quality of the microbiological data, quality assurance and reproducibility of the experimental data, as well as the availability and quality of the technical and experimental conditions were reviewed. Only those studies where process and experimental conditions were well documented were used.

2.2. Experimental conditions

The inactivation of micro-organisms by UV irradiation has been studied under different experimental conditions. Many studies used a collimated beam apparatus (CB tests) under bench-scale and well-defined laboratory conditions. A volume of inoculated water is irradiated during varying periods of time under a lamp emitting UV light. Other studies used continuous flow systems (CF systems) in a laboratory, in a pilot- or demonstration plant or under full-scale conditions where the water passes a reactor with one or more UV lamps and UV irradiation and contact time vary over the reactor.

2.3. Drinking water and wastewater studies

UV disinfection is applied in the drinking water industry and for disinfection of treated wastewater. Studies from both applications were reviewed and identified as such. Papers on wastewater studies (WWS) usually describe disinfection of secondary effluent with or without an additional pre-treatment. In the drinking water studies (DWS), water is generally of much lower turbidity and higher UV transmission than in wastewater. When relevant, the influence of these parameters is discussed.

2.4. UV fluence data

UV fluence cannot be measured directly, so it has to be inferred from monitoring the UV irradiance with a UV sensor and the time that the micro-organisms are exposed to UV. For the collimated beam experiments, the average UV irradiance and contact time are well-characterised and have small confidence intervals. For CF systems, the average UV fluence can be calculated from the same parameters. However, the confidence intervals are much larger, due to the much larger variation in contact time and in UV irradiation at different points in the reactor, compared to the collimated beam experiments. By modelling the hydraulic retention time in the UV reactor, using computational fluid dynamics (CFD) accuracy of these calculations has increased the last few years. Alternatively, the REF of CF systems can be determined with biodosimetry (Qualls and Johnson, 1983a; Sommer et al., 1999; Österreichisches normungsinstitut, 1999; Hoyer, 2004; USEPA, 2003). Biodosimetry is performed by challenging the UV reactor with a micro-organism with calibrated UV inactivation kinetics (biodosimeter) assessed with CB tests in the test water. With the measured inactivation of the biodosimeter and the calibration curve, the REF (mJ/cm^2) can be calculated.

2.5. Inactivation kinetics of the micro-organisms

The inactivation kinetics of a large number of pathogenic micro-organisms and indicator micro-organisms that are significant to the microbial safety of water have been calculated from studies where UV fluence has been determined under optimal conditions: CB tests with “drinking water” (low turbidity and high UV transmission).

Inactivation by UV is based on the damage caused to the nucleic acids (DNA/RNA) of the cell or virus. Primarily the formation of pyrimidine dimers, but also of other photoproducts of nucleic acids and nucleic acid lesions (von Sonntag et al., 2004), inhibit replication and transcription and hence, prevent the cell or virus from multiplying. The UV absorbance of DNA peaks around 260 nm; at lower and higher wavelengths the absorbance decreases. Below 230 nm the absorbance increases again. Most studies used low-pressure mercury lamps with a major wavelength output (85%) at 254 nm (monochromatic (MC) UV radiation) but for some micro-organisms the UV inactivation was (also) determined with polychromatic (PC) UV radiation from medium pressure (MP) lamps.

The UV sensitivity of the selected micro-organisms is described by the parameters of the inactivation kinetics. Inactivation is defined as the reduction of the concentration of culturable micro-organisms N due to the exposure to a concentration disinfectant C during a specific contact time t. The inactivation kinetic for chemical disinfectants is most

commonly described by the first-order disinfection model of Chick (1908) and Watson (1908) and the same model can be applied for UV disinfection. The inactivation of micro-organisms is usually described by the log inactivation of N. Based on the first-order model, the linear relationship between log inactivation and the UV dose or fluence is described by:

$$^{10}\log\left(\frac{N_t}{N}\right) = -k^* \text{ Fluence}, \quad (1)$$

where N_t is the microbial concentration after contact time t. Fluence is the product of the UV fluence rate (mW/cm^2) and the exposure t (mWs/cm^2 = mJ/cm^2). In the literature, two main deviations from first-order UV disinfection kinetics have been observed. Some authors (Knudson, 1985; Hoyer, 1998; Sommer et al., 1998; Mamane-Gravetz and Linden, 2005) observed no inactivation of bacteria or bacterial spores at low UV fluences followed by a normal log-linear relationship at higher UV fluences. This can be described by a shoulder model and is presented by the following equation:

$$\text{DI} = -k^* \text{ Fluence} - b, \quad (2)$$

where DI is the decimal inactivation $^{10}\log(N_t/N)$, b is the y-intercept, a negative value since the curve is crossing the fluence axis at the UV fluence where log-linear relationship starts (offset). The second deviation from the linear kinetics is no further increase in inactivation at high fluences, called tailing. Tailing is excluded in the k-value calculation in this review, by excluding the inactivation data at higher fluences in studies where tailing was observed (from the plots of inactivation versus fluence).

2.6. Influence of process conditions

The inactivation kinetics can be used to determine the disinfection efficacy or MIC (log) of full-scale UV systems and to assess the fluence requirement to obtain a certain MIC. However, for translation of CB results to full-scale UV systems it is essential to know the effect of process conditions on the efficiency of the radiation process. In contrast to oxidative disinfection processes with chemicals like chlorine and ozone, the efficacy of UV disinfection is not affected by conditions like temperature, pH (Severin et al., 1983; USEPA, 2003) and reactive organic matter. UV absorbance by organic and inorganic matter is included in the UV fluence calculation. But the following factors may affect the efficiency of UV disinfection at full-scale:

- Factors related to the micro-organisms: physiological state (pre-culturing, growth phase), strain diversity, repair mechanisms and particle association;
- Factors related to the fluence assessment: fluence distribution due to the distribution of the hydraulic retention time, adsorption, reflection and refraction of UV light through the water and lamp intensity (aging and fouling).

Several studies have addressed these aspects and are discussed to determine whether adaptation of the required fluence for a certain MIC, calculated by applying the UV sensitivity data to full-scale UV systems, is required and, if possible, to quantify to what extent.

3. Results and discussion

3.1. UV sensitivity of micro-organisms

The UV sensitivity or of the micro-organisms is described with the inactivation rate constant k (cm^2/mJ). A UV-sensitive micro-organism has a high k-value and requires a low fluence for inactivation according to Eq. (1).

3.1.1. Viruses and bacteriophages

The number of studies where UV sensitivity of specific pathogenic viruses and bacteriophages is determined under well-defined laboratory conditions with collimated beam apparatus (CB tests) ranged from 1 to 6. The total number of data per virus or bacteriophage ranged from 3 up to 109 (Tables 1 and 2). The calculated k-value (no shoulder; intercept = 0) showed a narrow 95% confidence interval (CI) and a high goodness-of-fit (13 out of 18 $r^2 > 0.85$). The six authors describing inactivation of seeded Poliovirus type 1 yielded a total of 61 data points presented in Fig. 1. The inactivation rate constant k calculated for a UV fluence range of 5–50 mJ/cm^2 was 0.135 (95%-CI = 0.007; $r^2 = 0.79$). Due to the observed tailing by Sommer et al. (1989) and Maier et al. (1995), MIC_{max} (the maximum observed MIC) is set at 5.4 log ($\geqslant$50 mJ/cm^2).

In Fig. 1, the fluence–response curves for Adenoviruses serotypes (ST)2, 15, 40 and 41 are also presented. These data demonstrate that the UV sensitivity of these serotypes for MC UV radiation show small differences. Adenovirus is the most persistent virus type presented in Table 1. This conclusion is supported by the recently published study of Nwachuku et al. (2005) who found k-values for serotypes 1 and 6 in the same order of magnitude as for the types presented in Table 1. Thurston-Enriquez et al. (2003) found the lowest k-value of 0.018 cm^2/mJ with Adenovirus ST40. Malley et al. (2004) determined the UV sensitivity of Adenovirus ST2 and ST41 for PC UV radiation (MP lamps). Up to a UV fluence of 90 mJ/cm^2, the UV sensitivity was a factor of 1.7 higher than that observed for MC UV radiation (low pressure lamps) (Table 1), but above this fluence they observed tailing (Fig. 1). By using bandpass filters they distinguished the germicidal effect of different wavelengths in the PC UV light at fluence ranges up to 90 mJ/cm^2 and showed that at wavelengths of 220 and 228 nm UV was significantly (a factor of 5–7) more effective in inactivating Adenovirus ST2 than UV light with a wavelength of 254 nm.

The fluence–response data and lines for Rotavirus type SA11 and for three types of Caliciviruses are presented in Fig. 2. Again Sommer et al. (1989) showed no further increase in inactivation at fluences above 50 mJ/cm^2 and Malley et al. (2004) showed that MC UV radiation was less efficient than PC UV radiation for inactivation of rotavirus SA-11. Using MP lamps, the k-values were 1.7 times higher (Table 1). Caliciviruses from different non-human hosts (feline, canine and bovine) showed highest UV sensitivity for the bovine type

Table 1 – UV sensitivity of viruses for monochromatic (MC) and polychromatic (PC) UV radiation determined with collimated beam tests

	Studies (*n*)	UV fluence (mJ/cm^2)	UV	k^a (±95%CI; r^2)	MIC_{max} (log)
Poliovirus type 1	6 (61)[b,c,d,e,f,g]	5–50	MC	0.135 (0.007; 0.79)	5.4
Adenovirus ST2,15, 40, 41	5 (98)[g,h,i,j,k]	8–306	MC	0.024 (0.001; 0.87)	6.4
Adenovirus ST40	1 (29)[i]	8–184	MC	0.018 (0.004; 0.88)	3.0
Adenovirus ST2, 41	1 (18)[k]	30–90	PC	0.040 (0.003; 0.77)	4.3
Rotavirus SA-11	5 (55)[b,d,e,k,l]	5–50	MC	0.102 (0.006; 0.78)	4.1
Rotavirus SA-11	1 (11)[k]	5–30	PC	0.154 (0.011; 0.92)	4.6
Calicivirus feline, canine	3 (29)[i,m,n]	4–49	MC	0.106 (0.010; 0.67)	5.5
Calicivirus bovine	1 (20)[k]	4–33	MC	0.190 (0.008; 0.96)	5.7
Calicivirus bovine	1 (20)[k]	2–15	PC	0.293 (0.010; 0.97)	5.9
Hepatitis A	3 (13)[e,l,o]	5–28	MC	0.181 (0.028; 0.70)	5.4
Coxsackie virus B5	2 (12)[h,l]	5–40	MC	0.119 (0.006; 0.97)	4.8

[a] Linear regression, intercept = 0.
[b] Chang et al. (1985).
[c] Harris et al. (1987).
[d] Sommer et al. (1989).
[e] Wilson et al. (1992).
[f] Maier et al. (1995).
[g] Meng and Gerba (1996).
[h] Gerba et al. (2002).
[i] Thurston-Enriquez et al. (2003).
[j] Thompson et al. (2003).
[k] Malley et al. (2004).
[l] Battigelli et al. (1993).
[m] De Roda Husman et al. (2003).
[n] Duizer et al. (2004).
[o] Wiedenmann et al. (1993).

Table 2 – UV sensitivity of bacteriophages for monochromatic (MC) and polychromatic (PC) UV radiation determined with collimated beam tests

	Studies (*n*)	UV fluence (mJ/cm^2)	UV	k^a (±95%CI; r^2)	MIC_{max} (log)
MS2-phages	5 (109)[b,c,d,e,f,g]	5–139	MC	0.055 (0.002; 0.93)	4.9
MS2-phages	1 (11)[f]	12–46	PC	0.122 (0.009; 0.92)	5.3
φX174	4 (30)[e,h,i,j]	2–12	MC	0.396 (0.025; 0.85)	4.0
PRD1	1 (4)[d]	9–35	MC	0.128 (0.014; 0.98)	3.8
B40-8	1 (14)[k]	1–39	MC	0.140 (0.010; 0.96)	5.6
T7	1 (3)[k]	5–20	MC	0.232 (0.080; 0.90)	4.6
Qβ	1 (5)[k]	10–50	MC	0.084 (0.003; 0.99)	4.2

[a] Linear regression, intercept = 0.
[b] Havelaar et al. (1990).
[c] Wilson et al. (1992).
[d] Meng and Gerba (1996).
[e] Sommer et al. (1998).
[f] Malley et al. (2004).
[g] Mamane-Gravetz et al. (2005).
[h] Battigelli et al. (1993).
[i] Oppenheimer et al. (1993).
[j] Sommer et al. (2001).
[k] Clancy et al. (2004).

(Malley et al., 2004) and the same study showed a 1.5 times higher inactivation with PC UV radiation compared to monochromatic UV. The *k*-value for inactivation of feline Calicivirus (0.106 cm^2/mJ) was in the same order of magnitude as that observed for Rotavirus, Poliovirus and Coxsackie virus B5 (Table 1). Hepatitis A virus was more sensitive to UV radiation.

Noroviruses are part of the human caliciviruses and are not culturable. Using RT-PCR, Watercare (2002) determined that environmental Norovirus was less effectively inactivated by UV than the other viruses as determined with culture assays. In their wastewater treatment plant, 10 samples before and 10 samples after UV were analysed; seven samples before UV and one sample after UV were positive for Norovirus. The

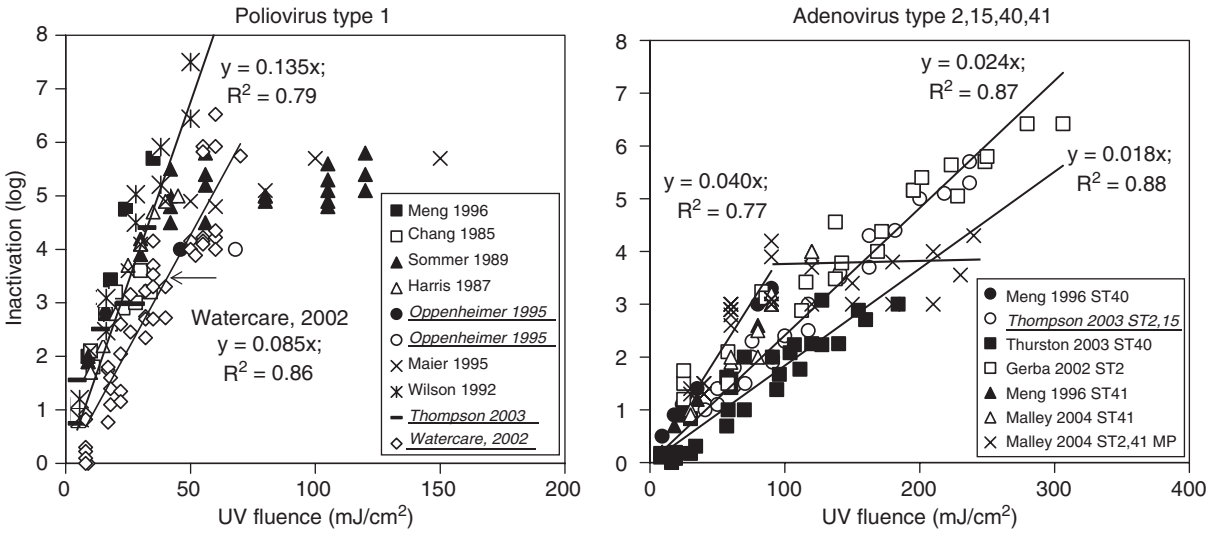

Fig. 1 – UV fluence–response curves for Poliovirus and Adenovirus (regular font DWS, *italic: WWS*).

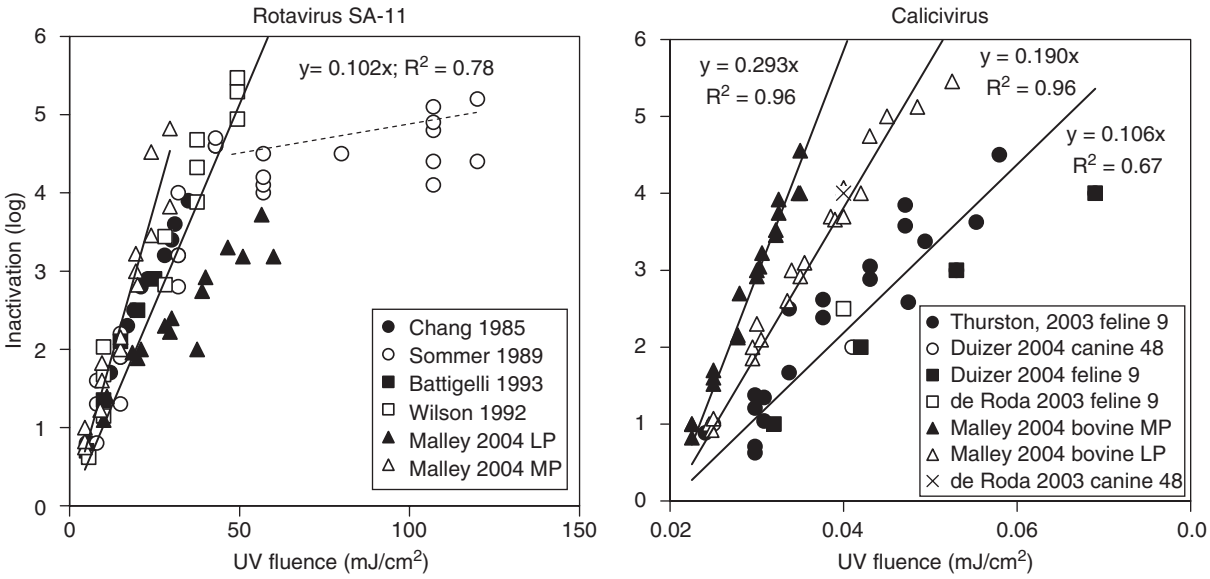

Fig. 2 – UV fluence–response curves for rotavirus and calicivirus.

mean fluence was 23 mJ/cm^2. From these (presence/absence) data, a 0.8 log inactivation was estimated for a UV fluence of 20 mJ/cm^2. At higher fluences (40 and 70 mJ/cm^2) all samples were negative. However, it is uncertain to which degree inactivation assessed with RT-PCR is representative for inactivation assessed with infectivity assays.

UV sensitivity of bacteriophages used or proposed as model organisms for the assessment of the REF of a UV system on full-scale is also presented in Table 2. MS2 phage is the most persistent of the tested phages with a *k*-value of 0.055 cm^2/mJ. *k*-values of the other bacteriophages ranged from 0.128 cm^2/mJ for PRD1 up to 0.396 cm^2/mJ for PhiX174. The fact that the *k*-values of these micro-organisms are in the same order of magnitude as observed for most pathogenic viruses (compare Tables 1 and 2) supports the use of these micro-organisms as surrogates for virus inactivation by UV. Only Adenoviruses are more resistant to UV.

Recently, Mamane-Gravetz et al. (2005) demonstrated that MS2 is more (three times) sensitive to low wavelengths near 214 nm emitted by MP lamps compared to 254 nm output of LP lamps, an observation in line with those of Malley et al. (2004) for Adenoviruses as described before. The *k*-values determined by Mamane-Gravetz et al. (2005) at wavelengths of 254 and 214 nm were 0.055 and 0.161 cm^2/mJ, respectively. These values are in the same order of magnitude as the *k*-values calculated in this study for LP and MP lamps, respectively (Table 2). The UV fluence of the MP lamp in the Malley study was calculated based on the average irradiance measured by a UV sensor and weighted by a germicidal factor at each wavelength (based on the DNA absorbance, relative to 254 nm). Thus, the fluence of LP and MP lamps was compensated for the wavelengths emitted by these lamps. Malley et al. (2004) argued that this weighting may have been biased for MP lamps. On the other hand, their results may

indicate a higher inactivation efficiency of MP lamps compared to LP lamps, a conclusion supported by the observations of Mamane-Gravetz et al. (2005).

3.1.2. *Bacteria and bacterial spores*

Bacteria (vegetative cells) are significantly more susceptible to UV radiation than viruses and therefore less extensively studied. In Fig. 3, fluence–response curves for some selected pathogenic bacteria are presented. With the exception of *E. coli* in five studies, only one or two studies were found for individual pathogenic bacteria. Wilson et al. (1992) tested the UV sensitivity of seven of the 10 bacterial species presented in Table 3. The number of data points ranged from 4 up to 41 for *E. coli*. The *k*-values varied from 0.312 cm^2/mJ for *Streptococcus faecalis* to 1.341 cm^2/mJ for *Vibrio cholerae* (both Wilson et al., 1992). Linear regression analysis showed low variation (95% confidence interval) and high goodness-of-fit (r^2). Sensitivity of *Legionella pneumophila* published in literature was highly variable. From the data of Antopol and Ellner (1979) and Wilson et al. (1992) a *k*-value of 1.079 and 0.400, respectively, was determined (Table 3). Knudson (1985) published a higher sensitivity ($k = 1.916$) and the *k*-value presented by Oguma et al. (2004) was 0.62. The latter author also demonstrated that the sensitivity of both *L. pneumophila* and *E. coli* to MC and PC was similar (Table 3).

Aerobic spores of *Bacillus subtilis* and anaerobic spores of *Clostridium perfringens* are clearly less sensitive to UV than the vegetative bacterial cells (Table 3) and also most of the viruses and phages (Tables 1 and 2). The data on the UV sensitivity of *Clostridium perfringens* were derived from a CF system with MP lamps, in which the UV fluences were determined with biodosimetry (REF) with UV calibrated spores of *B. subtilis*.

3.1.3. *Pathogenic protozoa*

Interest in UV as a disinfection process for water has increased after Clancy et al. (1998) showed that *Cryptosporidium parvum* oocysts were highly susceptible to UV when the effect on the infectivity was assessed with the neonatal mouse model. Since then, Clancy and several other authors have studied inactivation of *Cryptosporidium parvum* and *Giardia muris* by UV radiation (Table 4). Figures 4 and 5 show substantial inactivation of (oo)cysts of both protozoa at low UV fluences (<20 mJ/cm^2) by LP and MP lamps. Recently, Johnson et al. (2005) demonstrated a similar UV sensitivity for *C. hominis* oocysts which predominates in human cryptosporidiosis infections. Based on the regression analysis of these fluence–response data, efficacy of LP and MP lamps for oocyst inactivation is in the same order of magnitude (Table 4). Comparison of these *k*-values with the *k*-values from Tables 1 and 2 shows that these protozoa are more sensitive to UV than viruses, but less sensitive compared to most bacteria. The regression analysis of the accumulated data shows a low goodness-of-fit ($r^2 = 0.37$; 0.49 and 0.81) and positive intercept values. Furthermore, Craik et al. (2000, 2001) observed considerable tailing for a number of inactivation data at high UV fluences (Fig. 4c). Qian et al. (2004) described the protozoan data with a statistical method (Bayesian meta-analysis) which resulted in the UV fluence requirement curves presented in Figs. 4 and 5 (USEPA, 2003). These curves were calculated for an inactivation requirement of up to 3 log and could be described by a log–log relationship (log inactivation of *Giardia* = 1.2085 LN UV fluence + 0.0715; $r^2 = 0.99$; log inactivation of *Cryptosporidium* = 1.2344 LN UV fluence—0.1283; $r^2 = 0.99$).

One study described the inactivation of *Acanthamoeba* spp. by UV in CB tests using CD1 neonatal mouse model test to measure infectivity (Maya et al., 2003). Just as observed for *B. subtilis*, an offset UV fluence is required for this organism to see an effect on infectivity of these pathogens. This offset value of 30 mJ/cm^2 as well as the low *k*-value of 0.021 calculated from the successive log-linear relationship, show that the sensitivity of this micro-organism and of the most resistant virus type Adenovirus to UV are in the same order of magnitude.

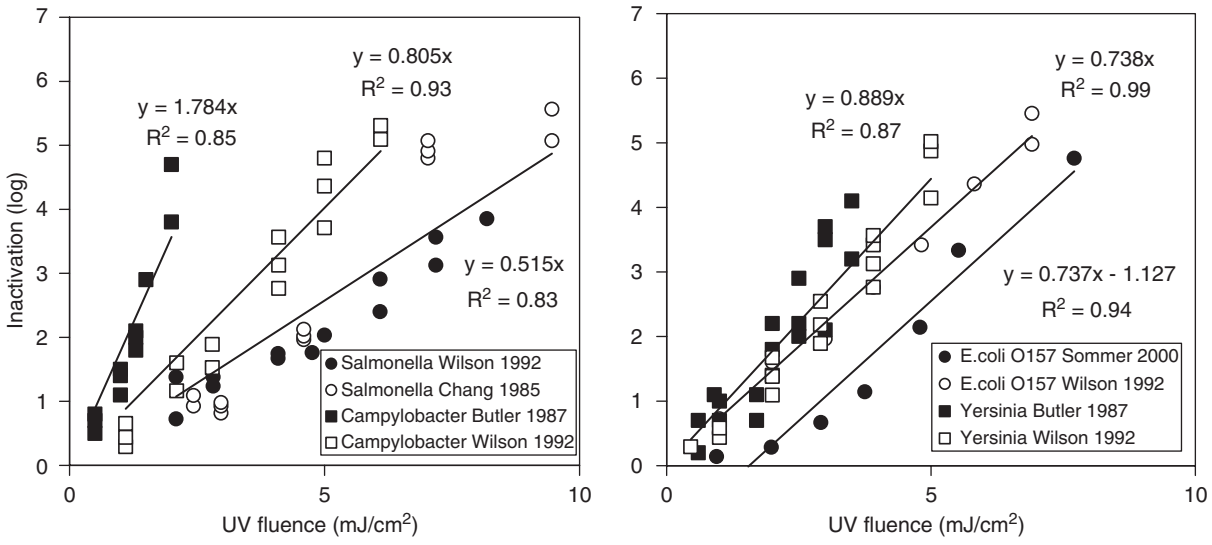

Fig. 3 – UV fluence–response curves for pathogenic bacteria.

Table 3 – UV sensitivity of bacteria and bacterial spores for monochromatic (MC) and polychromatic (PC) UV radiation determined with collimated beam tests

	Studies (data)	Fluence (mJ/cm^2)	UV	k (±95%CI; r^2)	Offset[a] (mJ/cm^2)	MIC_{max} (log)
Salmonella typhi	2 (26)[b,c]	2–10	MC	0.515 (0.047; 0.83)	0	5.6
Campylobacter jejuni	2 (27)[c,d]	0.5–6	MC	0.880 (0.124; 0.65)	0	5.3
Yersinia enterocolitica	2 (34)[c,d]	0.6–5	MC	0.889 (0.060; 0.87)	0	5.0
Shigella dysenteriae	1 (9)[c]	1–5	MC	1.308 (0.087; 0.95)	0	5.9
Shigella sonnei	1 (9)[b]	3–8	MC	0.468 (0.053; 0.89)	0	4.7
Vibrio cholerae	1 (10)[c]	0.6–4	MC	1.341 (0.113; 0.94)	0	5.8
Legionella pneumophila	1 (15)[c]	1–12	MC	0.400 (0.040; 0.92)	0	4.4
Legionella pneumophila	1 (4)[e]	0.5–3	MC	1.079 (0.077; 0.99)	0	3.0
Escherichia coli O157	2 (16)[c,f]	1–7	MC	0.642 (0.082; 0.85)	0	5.5
Escherichia coli	6 (41)[b,d,g,h,i,j]	1–15	MC	0.506 (0.049; 0.71)	0	6.0
Escherichia coli	1 (23)[k]	1.5–9	PC	0.539 (0.070; 0.64)	0	5.2
Streptococcus faecalis	2 (19)[b,g]	2.5–16	MC	0.312 (0.032; 0.85)	0	4.6
Bacillus subtilis	4 (30)[b,h,l,m]	5–78	MC	0.059 (0.007; 0.91)	12.3	4.0
Clostridium perfringens	1 (9)[m]	48–64	PC	0.060 (0.027; 0.81)	18	3.0

[a] Offset is threshold-value >0: linear regression with intercept≠0.
[b] Chang et al. (1985).
[c] Wilson et al. (1992).
[d] Butler et al. (1987).
[e] Antopol and Ellner (1979).
[f] Sommer et al. (2000a).
[g] Harris et al. (1987).
[h,i] Sommer et al. (1989, 1996a).
[j] Zimmer and Slawson, (2002).
[k] Oguma et al. (2002).
[l] Sommer et al. (1998).
[m] Hijnen et al. (2004b) (continuous flow system).

Table 4 – UV sensitivity of protozoa and *Acanthamoeba* spp. for monochromatic (MC) and polychromatic (PC) UV radiation determined with collimated beam tests

	Studies (data)	k (±95% CI; r^2)	Range (mJ/cm^2)	Intercept (95%)	MIC_{max}
C. parvum	6 (38)[a,b,c,d,e,f]	0.243 (0.08; 0.49)	0.5–6.1; PC	1.502 (0.538)	3.0
C. parvum	4 (65)[a,c,f,g]	0.225 (0.07; 0.37)	0.9–13.1; MC	1.087 (0.403)	3.0
Giardia muris	1 (4)[h]	0.122 (0.178; 0.81)	1.5–11; MC	1.303 (1.280)	2.4
Giardia lamblia	1 (2)[i]	nd	0.05–1.5; MC	nd	2.5
Acanthamoeba spp.	1 (16)[j]	0.021 (0.004; 0.94)	43–172; MC	0.499 (0.449)	4.5

[a,b] Clancy et al. (2000, 2002).
[c] Craik et al. (2001).
[d] Shin et al. (2001).
[e] Morita et al. (2002).
[f] Rochelle et al. (2004).
[g] Bolton et al. (1998).
[h] Craik et al. (2000).
[i] Linden et al. (2002).
[j] Maya et al. (2003).

3.2. *Process conditions*

The k-values summarised in Tables 1–3 can be used to determine the scale of new full-scale UV treatment processes or to calculate the inactivation efficiency of operational UV systems. Translation of UV sensitivity assessed with CB tests and seeded micro-organisms to the efficiency of UV disinfection under full-scale conditions, however, is influenced by factors related to the micro-organisms and by factors related to the fluence assessment. This is similar to the translation of lab-scale tests for chemical disinfection to full scale, as illustrated for ozone by Smeets et al. (2004, 2005). The literature on the influence of these factors has been reviewed. For pathogenic micro-organisms two studies were found (Fig. 6) that investigated the inactivation of environmental pathogenic micro-organisms under different conditions. For indicators, more studies were available. Most of the evaluated data came from WWS and to a lesser extent from DWS. Inactivation of seeded or environmental indicator micro-organisms (coliforms, enterococci, clostridia spores, F-specific

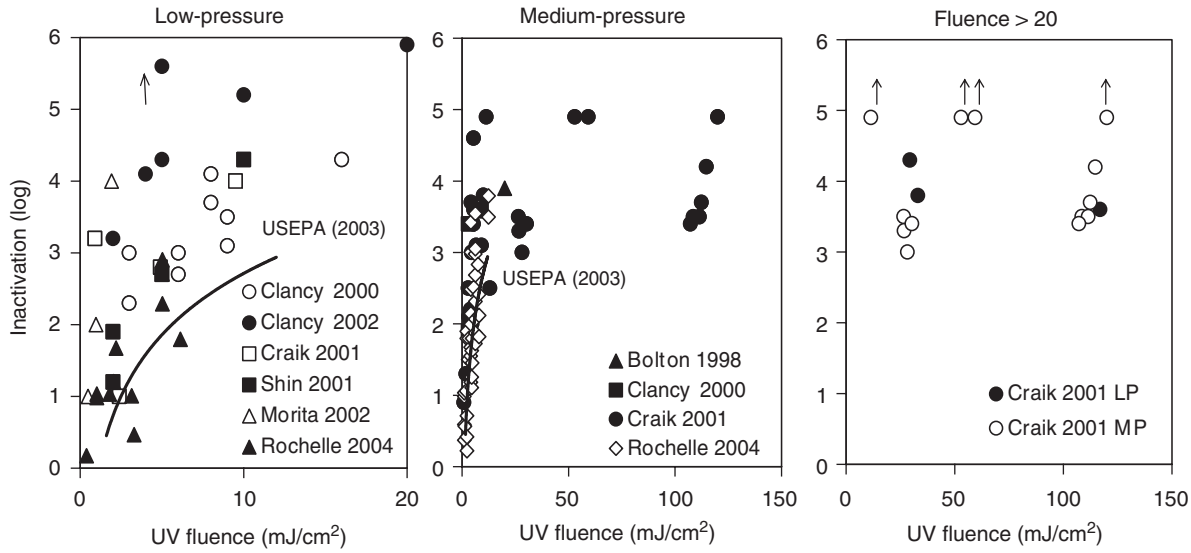

Fig. 4 – UV fluence–response curves for *Cryptosporidium parvum* (multiple strains) and LP and MP lamps (a, b) and (c) tailing in the inactivation data observed by Craik et al. (2001) for fluences above 20 mJ/cm^2.

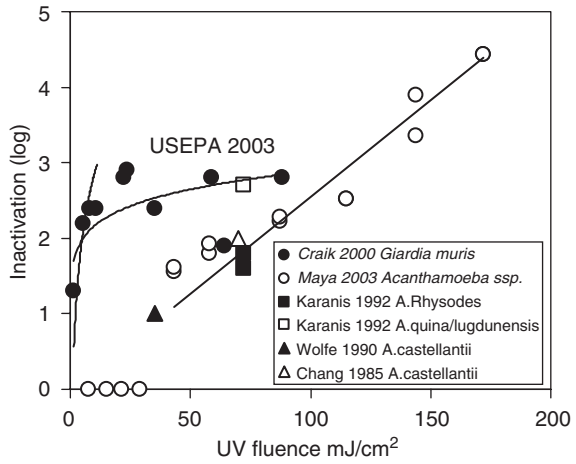

Fig. 5 – UV fluence–response curves for *Giardia muris* and *Acanthamoeba* spp.

RNA (FRNA) phages, *Bacillus* spores) has been determined in either CB apparatus or CF systems. Inactivation data are presented in Figs. 7–11, where CB test results are separated from results from CF systems. The findings are reviewed in the two following paragraphs.

3.2.1. *Micro-organism-related factors*

The UV sensitivity of seeded and environmental micro-organisms is compared in some studies under identical conditions or by comparing results from a study with environmental organisms with the overall data for seeded organisms tested in CB apparatus or CF systems (DWS, Figs. 6–9). Environmental *Salmonella*, faecal coliforms and enterococci in CB tests in wastewater (Maya et al., 2003) were more resistant to UV light than the seeded micro-organisms of the same species (DWS, Figs. 6, 8 and 9).

A higher UV resistance of environmental spores compared to seeded spores (which were surviving isolates from the environmental spores) was also observed for *Bacillus* spp. (Mamane-Gravetz et al., 2005; CB tests) and sulphite-reducing clostridia SSRC (Gehr et al., 2003; CB test and Hijnen et al., 2004b; Watercare, 2002; CF systems), (Fig. 10). A higher resistance to UV of environmental bacteria is also demonstrated by the inactivation data of thermotolerant coliforms in the WWS studies of Watercare (2002) and Gehr and Nicell (1996) as shown in Fig. 8. Based on the difference in k-value between the Watercare data and the DWS data assessed in CB tests, the k-value decreased a factor of seven (from 0.506 to 0.066; Fig. 8).

In a study with a CF system (LP lamps) operated at a fluence of 25 mJ/cm^2, complete inactivation of environmental Poliovirus (type 1) in a chalk well water was observed with an estimated inactivation of more than 2.3 log (Slade et al. 1986; 21 samples of 0.15–1 m^3 tested over a period of 1 year). This might indicate little or no difference in sensitivity between environmental and lab-cultured Polioviruses; the latter are inactivated with 3.4 log at this fluence calculated from the k-value of Table 1. In the WWS study of Watercare (Watercare, 2002; Simpson et al., 2003; Jacangelo et al., 2003), the UV sensitivity of environmental FRNA was comparable to the UV sensitivity of seeded MS2 phages tested under similar conditions (CB tests; Fig. 7). A k-value of 0.049 cm^2/mJ was calculated for the seeded MS2-phages while for environmental FRNA tested under similar conditions in CB tests resulted in a k-value of 0.047 cm^2/mJ (Fig. 7). In contrast, a lower UV resistance of environmental Adenoviruses was observed in a CF system (Watercare study) compared to seeded Adenoviruses tested in DWS studies (CB tests, Fig. 6). Because 35% of the observations in this study yielded a higher inactivation than could be detected (>2.7 up to >3.3 log at a fluence range of 23 up to 50 mJ/cm^2; Fig. 6), the difference is even larger; these data have not been used in the k-value calculation

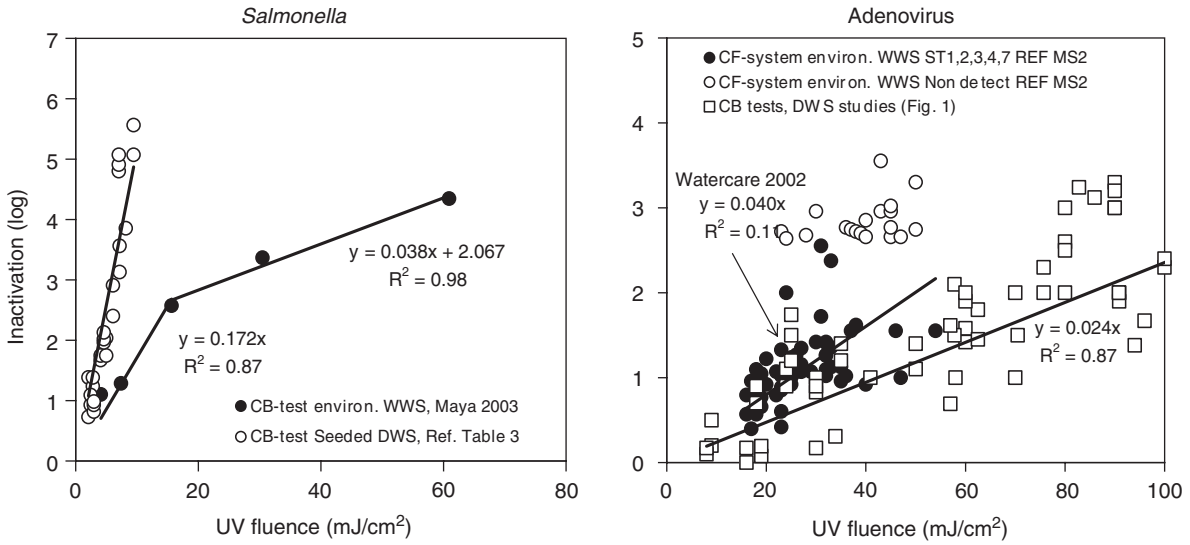

Fig. 6 – Comparison of UV fluence–response curves for seeded and environmental *Salmonella* and Adenoviruses.

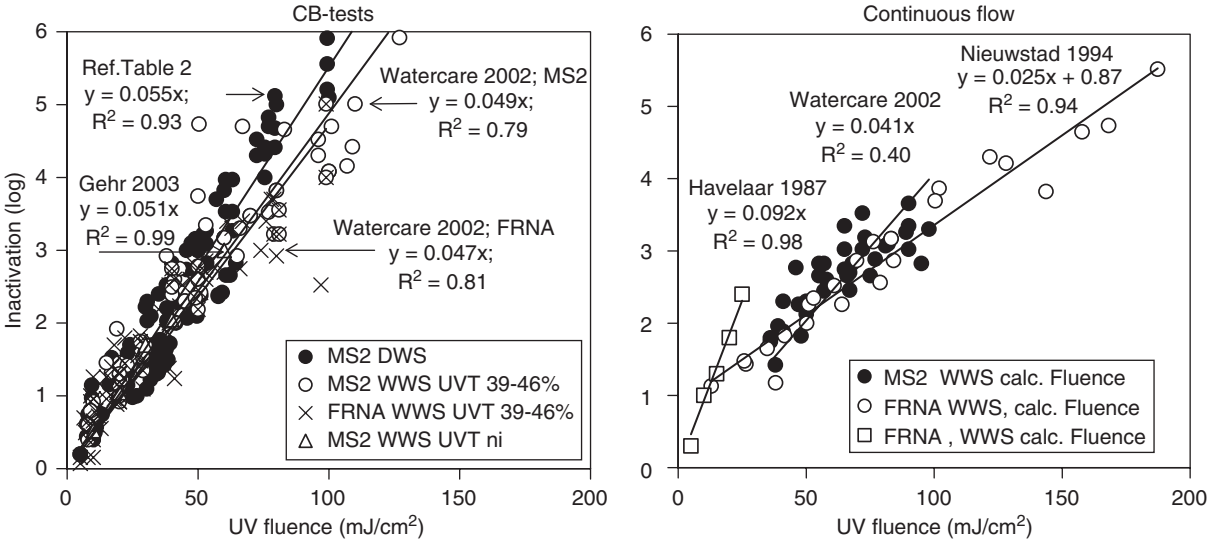

Fig. 7 – UV fluence–response curves for seeded MS2 FRNA phages and environmental FRNA phages determined under different conditions (ni = no information; Nieuwstad and Havelaar, 1994).

presented in Fig. 6. Predominant Adenovirus types in the Watercare study were serotypes 1–4 and 7, with less commonly serotypes 5, 8, 11, 13, 15, 19, 25 and 29. The higher susceptibility of the environmental Adenovirus in this study could be the result of the absence of Adenovirus, type 40, the most persistent serotype.

Overall, increased UV resistance of environmental micro-organisms was more explicit for the bacterial spores and for vegetative bacteria, and was of less significance for FRNA phages and viruses. To the authors knowledge, no data have been published on the UV sensitivity of environmental (oo)cysts of *Cryptosporidium* and *Giardia*. The observed difference in UV sensitivity for bacteria and spores may be attributable to the physiological state of the micro-organisms, strain diversity, DNA-repair mechanisms and particle association. These factors are discussed in more detail below.

Physiological state

The physiological state of micro-organisms affects the sensitivity to environmental stress factors such as UV radiation. Martiny et al. (1990), Mofidi et al. (2002) and Malley et al. (2004) showed that UV sensitivity was related to the growth phase of the bacteria; with the highest sensitivity in the active growth phase and lower sensitivity in the stationary phase.

Strain variation

Different strains of one species may have different UV sensitivity, as demonstrated for *E. coli* by Sommer et al.

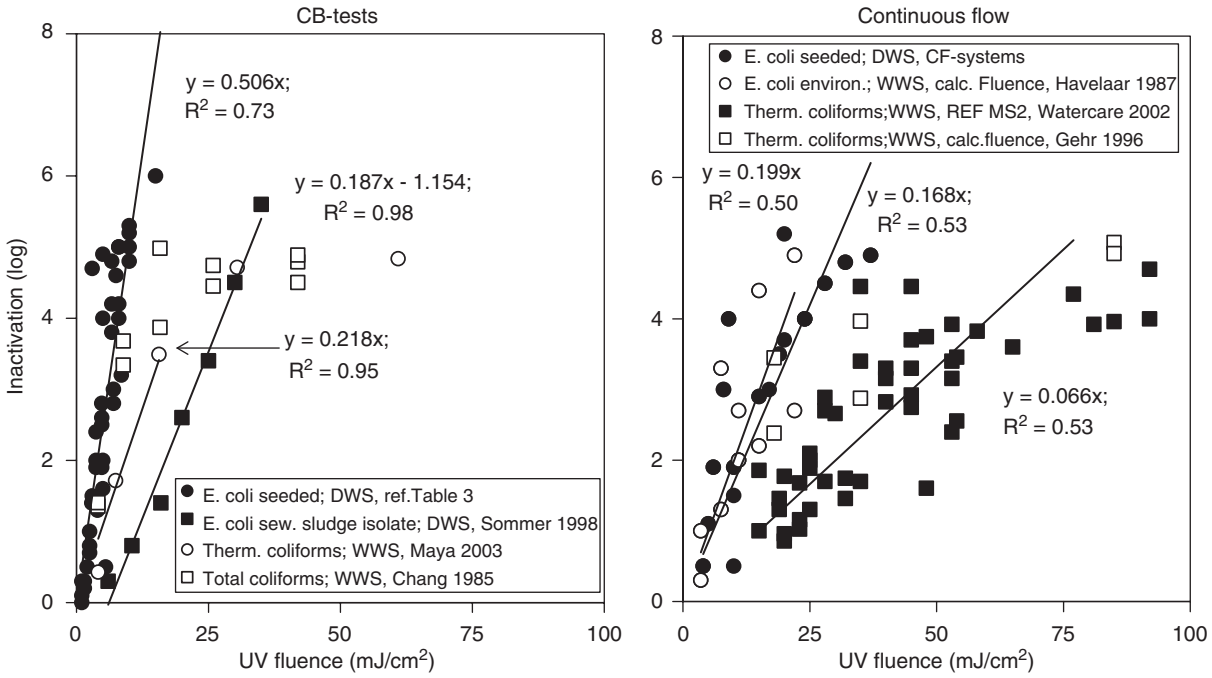

Fig. 8 – UV fluence–response curves for seeded and environmental coliforms (*E. coli*, thermotolerant coliforms and total coliforms) determined under different conditions.

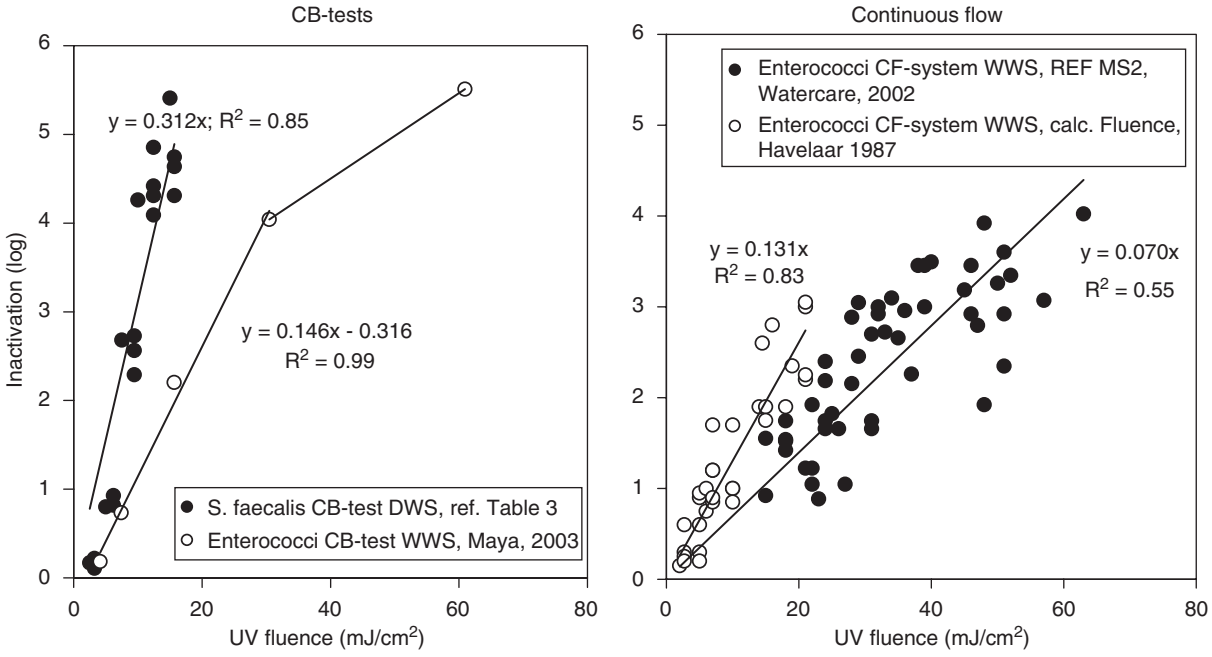

Fig. 9 – UV fluence–response curves for seeded and environmental enterococci determined under different conditions.

(1998, 2000a) (Fig. 8) and Malley et al. (2004). UV sensitivity of different *E. coli* strains in these studies varied by a factor of 5.8 and 3.7, respectively. The latter study demonstrated a higher sensitivity of *E. coli* O157:H7 compared to non-pathogenic/toxic strains. In contrast, Clancy et al. (2002) and Rochelle et al. (2004) showed that the high inactivation efficiency of UV radiation for *Cryptosporidium* was observed in multiple strains of *Cryptosporidium parvum*. The similar UV sensitivity observed for *C. hominis* (Johnson et al., 2005) suggests that this high sensitivity of *Cryptosporidium* oocysts is common for all sub-species.

Repair

Exposure to UV results in damage to the nucleic acids of the cell. Although also other components of the cell may be damaged by UV, micro-organisms may still retain metabolic functions such

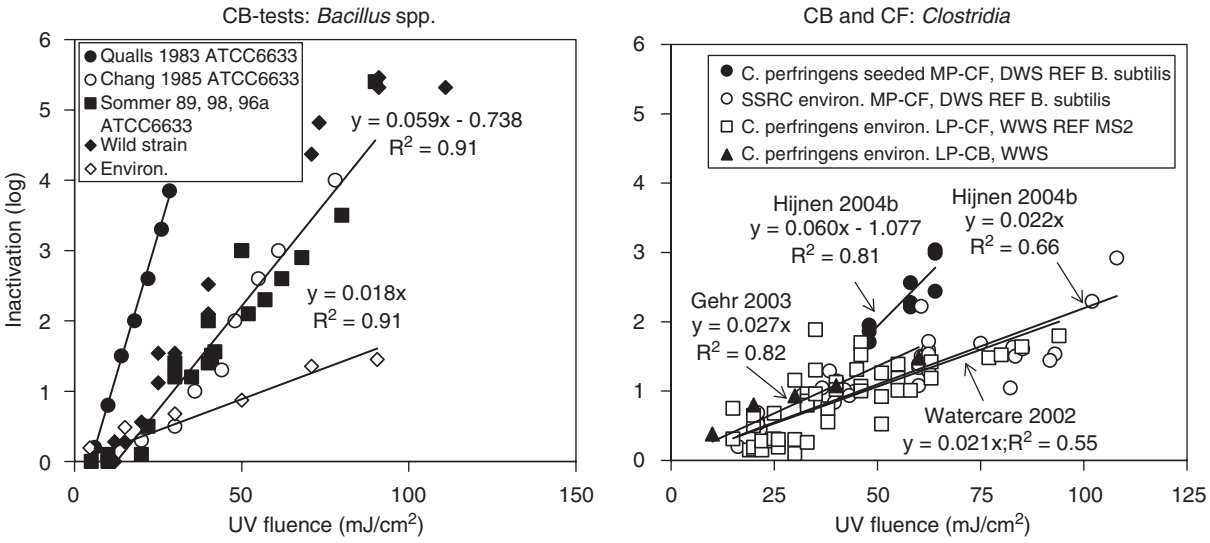

Fig. 10 – UV fluence–response curves for seeded and environmental bacterial spores determined under different conditions.

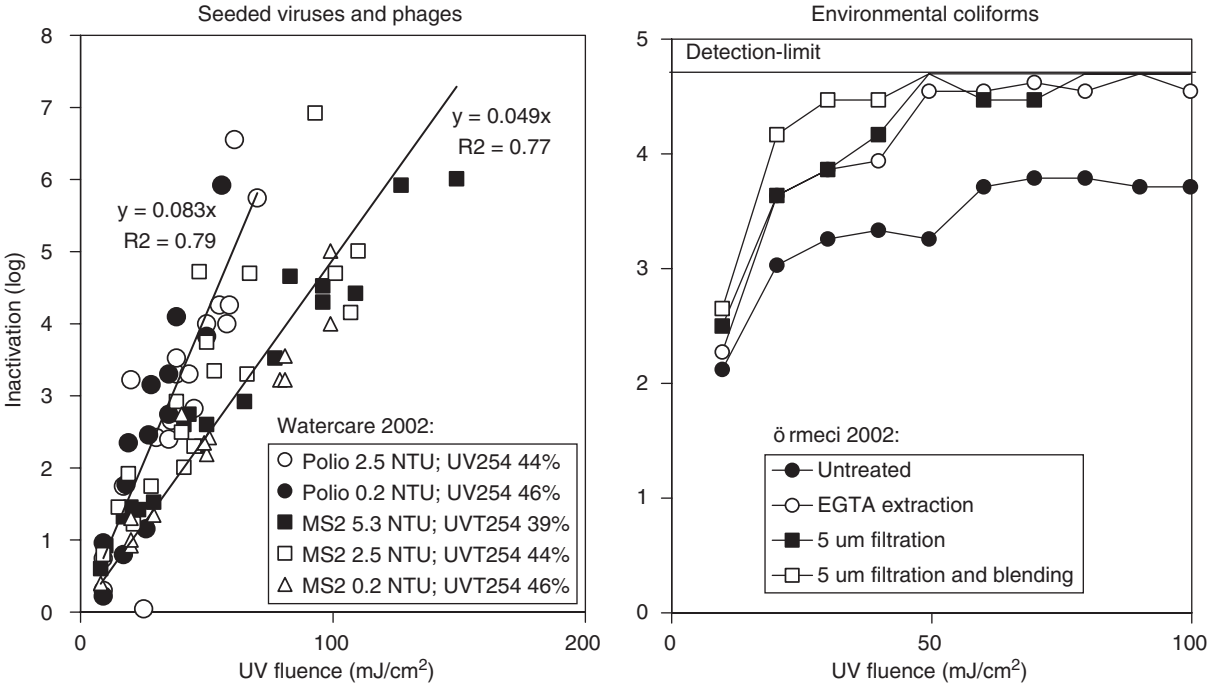

Fig. 11 – Effect of water quality and association with particles on UV fluence–response curves for viruses and phages and for environmental coliforms, respectively.

as enzyme activity. Over time, organisms have developed mechanisms to repair DNA damage as a result of exposure to UV from the sun. The mechanisms of repair are comprehensively described in von Sonntag et al. (2004). Two types of repair have been described: dark repair and photo-reactivation.

Dark repair does not require light and has been demonstrated in almost all bacteria. Spores have no active metabolism, but repair starts upon germination. Viruses have no metabolism so cannot repair damage to their genome themselves. However, several viruses have been shown to use the repair enzymes of the host cell. This is suggested as the cause of the high resistance of Adenovirus, a double-stranded DNA virus, which can use the host cell's repair mechanism, while RNA viruses may not. Some viruses even carry the genes for repair enzymes (Lytle, 1971; refs. von Sonntag), but this is not the case for viruses that are transmitted via water.

Photo-reactivation occurs in conditions of prolonged exposure to (visible) light and is specifically targeting pyrimidine dimers. For bacteria, several CB studies demonstrated repair after light exposure. The significance of this phenomenon to the required fluence to achieve a certain inactivation can be deduced from the influence of repair on the inactivation

kinetics (dose–response curves), but also from the occurrence of these repair mechanisms under conditions of disinfection practice.

Most photo-reactivation studies with CB tests used low fluences and optimal conditions for light exposure for repair (thin layer of fluid). The results show that under these conditions fluence requirement increases with increasing fluence (lower *k*-values). Quantitative data showed a 2.8–4.6 higher UV fluence requirement for 1–3 log inactivation of *L. pneumophila* (Knudson, 1985); based on these data *k*-value decreased a factor of 3.2. Oguma et al. (2004) observed a comparable log repair at an initial inactivation of 3 log after UV disinfection with LP and MP lamps and complete photo-reactivation. For *E. coli*, Bernhardt (1994) showed an increased offset value and decreased *k*-value. For several bacteria spp. (*E. coli*, *Yersinia enterocolitica*, *Salmonella typhi* and *Vibrio cholerae*), he calculated an increased fluence requirement for a 4 log inactivation ranging from a factor of 1.8 up to 4.2 to account for complete photo-reactivation. For *E. coli* similar enhancement of fluence requirement was observed by Hoyer (1998); 3.5 times for 4 log inactivation). Sommer et al. (2000a) showed a decrease in *k*-value after photo-reactivation and also demonstrated that dark repair is of less importance for *E. coli*. The latter observation was confirmed by Zimmer and Slawson, (2002), who demonstrated that photo-reactivation of *E. coli* did not occur after MP lamps, an observation also supported by Oguma et al. (2002, 2004).

Morita et al. (2002) demonstrated photo-reactivation and dark repair of DNA in *Cryptosporidium parvum* with the endonuclease-sensitivity site assay. The animal infectivity, however, was not restored. Furthermore, they concluded that UV radiated oocysts are able to excyst but have lost their ability to infect host cells. Similar observations were reported by Shin et al. (2001) and Zimmer et al. (2003). Craik et al. (2000) and Linden et al. (2002) came to the same conclusion for *G. muris* and *Giardia lamblia* cysts, respectively. Belosevic et al. (2001), however, showed the ability of DNA repair by some *Giardia* spp. after UV radiation with MP lamps. This was also presented by Kruithof et al. (2005); in vivo reactivation (dark repair) of *G. muris* cysts occurred at fluence values as low as 25 mJ/cm^2, but not at 60 mJ/cm^2, after prolonged time of incubation (3%, 14% and 20% reactivation after 10, 20 and 30 days, respectively). Also, DNA repair of *G. lamblia* cysts after exposure to MC UV irradiation is recently reported (Shin et al., 2005). An extensive study was presented on repair in *Cryptosporidium parvum* oocysts by Rochelle et al. in 2004. Identification of possible DNA-repair genes in *Cryptosporidium parvum* showed that the oocysts contain all of the major genetic components of the nucleotide excision repair complex. Nevertheless, inactivation displayed by oocysts immediately after UV exposure or displayed by oocysts after UV exposure followed by various repair conditions were generally in the same order of magnitude. This suggests that repair of UV induced damage in *Cryptosporidium parvum* after UV exposure in drinking water is not likely to occur.

Particle association

Higher resistance of particle-associated faecal bacteria has been observed in secondary effluents. This phenomenon was demonstrated by Qualls et al. (1983b) and Havelaar et al. (1987) for thermotolerant coliforms and the enterococci, respectively. A lower inactivation rate was observed in non-filtered effluent of sewage water plants compared to filtered samples (pore size 8 μm). Recently, Örmeci and Linden (2002) applied different techniques (extraction with EGTA, filtration of 5 μm filters with or without homogenisation by blending) to separate particle- and non-particle associated coliforms and showed an increased resistance of environmental coliforms associated with particles to UV (Fig. 11). Aggregates of *B. subtilis* spores were artificially made with clay in a Jar Test apparatus by Mamane-Gravetz and Linden (2004) and caused a reduction in inactivation efficiency. The *k*-value decreased from 0.0617 for the suspended spore-clay solution to 0.0579 cm^2/mJ for the aggregated spore-clay suspension. The same authors published a new study in 2005, in which they found evidence for a correlation of hydrophobicity of spores with aggregation. Aggregation may be a cause of tailing (no further increase of inactivation at higher fluence) observed in the kinetics. The *k*-values of isolated environmental *Bacillus* strains in the tailing phase of the kinetics were similar to the *k*-values of the original and natural *Bacillus* spore population. This indicates that a shielding effect of aggregation or particle association is a significant factor in the low susceptibility of environmental *Bacillus* spores, and also observed for the environmental clostridia spores as presented in Fig. 10.

3.2.2. *Fluence-related factors*

Variability in fluence may be caused by water quality (adsorption, reflection and refraction) and the distribution of the hydraulic retention time in CF systems.

Water quality

The presence of UV absorbing organic and inorganic compounds in water will reduce the UV fluence but results showed that fluence values can easily be corrected for the UV transmission of the water. Havelaar et al. (1990) placed 0.22 μm membrane filtered secondary effluent (UV transmission of 40–60%) in the UV pathway of the collimated beam apparatus and showed no decrease of the inactivation rate constant for MS2 phages after correction of the fluence for the transmittance. In studies with CF systems, both Schoenen et al. (1995) and Sommer et al. (1997) showed that the inactivation efficiency assessed at similar sensor readings was more sensitive for change in lamp intensity than for change in the water transmittance.

The influence of water quality on the efficiency of UV disinfection can be demonstrated by comparing results of DWS with WWS conducted under similar conditions. CB tests with seeded Polioviruses in secondary effluent with high turbidity and low UV transmission were published by Oppenheimer et al. (1995), Watercare (2002) and Thompson et al. (2003) (Fig. 1). In the first two studies, a lower inactivation was observed in wastewater than in DWS. In the Watercare study, the inactivation rate constant *k* of seeded Poliovirus in filtered secondary effluent was a factor 1.6 lower than the *k*-value calculated from the DWS (Fig. 1). The same study (Watercare, 2002) observed a slightly lower inactivation of MS2 in secondary effluent compared to the inactivation of MS2 in drinking water

Table 5 – Calculated UV fluence versus fluence assessed with biodosimetry (REF)

System	Study	Model organism	*n*	Ratio $UV_{calculated}$/REF (±SD)
A[a]	DWS	*B. subtilis*	3	1.33 (0.07)
B[a]	DWS	*B. subtilis*	3	0.81 (0.02)
C[a]	DWS	*B. subtilis*	3	0.79 (0.10)
D[a]	DWS	*B. subtilis*	3	1.00 (0.28)
E[a]	DWS	*B. subtilis*	3	0.73 (0.19)
F[a]	DWS	*B. subtilis*	3	0.62 (0.14)
G[b]	DWS	*B. subtilis*	9	0.59 (0.03)
H[c]	WWS	*MS2 phage*	37	0.83 (0.25)

[a] Sommer et al. (2000b).
[b] Hijnen et al. (2004b).
[c] Watercare (2002).

at the same fluence (Fig. 7). CB tests were conducted with secondary effluent with high and low turbidity (0.2–2.5 NTU and UVT of 40–68%). They demonstrated that there was no impact of turbidity on the inactivation of seeded Polioviruses and MS2 phages (Fig. 11). In addition, data from the WWS study of Thompson et al. (2003) showed no decreased inactivation of seeded Poliovirus and Adenovirus compared to inactivation of these organisms tested in DWS studies (Fig. 1).

Fluence determination in CF systems

In the literature, only few DWS have been published where fluence–response curves were determined with CF systems. Results from the studies of Martiny et al. (1990), Schoenen et al. (1991) and Bernhardt et al. (1992, 1994) showed lower inactivation rate constants for *E. coli* (Fig. 8) when compared to the *k*-value determined from CB tests. The fluence in the CF systems in these studies was based on information of the supplier of the UV equipment or on actinometry.

Information about the precision of fluence calculations can be obtained with biodosimetry. Spores of *Bacillus subtilis* and MS2 phages are used as model organisms in biodosimetry assays to assess the REF of CF systems. Sommer et al. (2000b) determined the REF with UV_{254} calibrated spores of *B. subtilis* of more than 30 commercially available CF systems and presented the results of six systems. In one system, the REF was equal to the UV fluence calculated according to the supplier's instructions, four systems showed that that REF was 19–38% lower than the calculated fluence and in one system REF was 33% higher than the calculated fluence (Table 5). The average ratio of the REF to the calculated fluence was 0.83 with a relatively high standard deviation of 0.25 (Table 5). The overestimation of the effective fluence (REF) by fluence calculation was supported by data presented by Hijnen et al. (2004b). In conclusion, calculated fluence data in CF systems frequently do not match those obtained by biodosimetry. Biodosimetry is essential to determine the efficacy of CF systems (DVGW, 1997; Sommer et al., 2000b; USEPA, 2003). The introduction of CFD for fluence calculations (no data presented) is improving the quality and precision of fluence calculations.

Reflection

Reflection caused by the construction materials of the UV reactor will have an influence on the inactivation efficiency determined by biodosimetry (Sommer et al., 1996b). This factor is of greater influence in single lamp systems than in multiple lamp systems because of the higher surface–volume ratio.

4. General discussion

4.1. *Kinetics of UV inactivation*

Most of the inactivation data can be adequately described with the first-order disinfection model, at least for a certain fluence range. An offset UV fluence before inactivation starts, i.e., a shoulder model, is observed for *Bacillus* spores and *Acanthamoeba* spp. The simple inactivation model, where the shoulder is given as an offset of the first-order model, is used in this study.

Another deviation from first-order kinetics is the reduction of inactivation rate at higher UV fluences (tailing). This is observed in several DWS with CB tests (Polioviruses, rotaviruses, *E. coli, C. parvum* and *G. muris*) and also for environmental bacteriophages and bacteria in WWS in CF systems. Tailing normally starts after at least 99% of the initial available micro-organisms are inactivated and is observed to a larger extent in the more UV susceptible micro-organisms. For the most resistant organisms (Adenoviruses, MS2 phages, bacterial spores and *Acanthamoeba* spp.), tailing was not observed. The cause of tailing is still under debate. Several causes have been hypothesised, such as experimental bias, hydraulics, aggregation of micro-organisms or a resistant subpopulation, but no conclusive evidence is available for any of these. For micro-organisms where tailing is observed, we have used the first-order model only for the fluence range that yielded a linear relation with the inactivation in the experiments. Because of the observed tailing, extrapolation of this inactivation rate to higher fluences is yielding uncertain results. For use in QMRA, the higher fluences can be assumed to yield (at least) the same inactivation credits as the highest fluence in the linear relation.

4.2. *Significance for water disinfection*

This study provides an extensive overview of the efficacy of UV disinfection for viruses, bacteria and bacterial spores and protozoan (oo)cysts, obtained from the reviewed literature. The *k*-values that were calculated from the reviewed studies

can be used in QMRA and treatment design to determine the efficacy of a UV fluence in the inactivation of the range of reported bacterial and viral pathogens and indicator organisms. For *Cryptosporidium* and *Giardia*, the logarithmic functions given in USEPA (2003) were used for calculating the inactivation efficacy.

Of the pathogens, viruses are generally more resistant than *Cryptosporidium*, *Giardia* and the bacterial pathogens. Adenovirus 40 is the most UV-resistant waterborne pathogen known. *Acanthamoeba* is also very resistant. Bacterial spores, especially environmental spores of *Clostridium* are also resistant to UV, with *k*-values that are comparable to the Adenoviruses (Fig. 10).

4.3. *Correction of the required fluence: micro-organism-related factors*

Based on the increased UV resistance observed for environmental *Salmonella*, enterococci, thermotolerant coliforms, FRNA phages and spores of sulphite-reducing clostridia, correction of the fluence requirement for inactivation of bacteria and bacterial spores from the environment seems appropriate. The evaluated studies suggest a two times increased fluence requirement for bacteria and four times for bacterial spores in drinking water. For wastewater this is most likely not enough and based on Fig. 8 a factor of seven seems more appropriate. The results of environmental polioviruses (Slade et al., 1986) and FRNA phages and Adenoviruses (Watercare, 2002) indicate that such a correction is not needed for phages and viruses. However, further research is needed to support these findings. Similarly, studies on the increased resistance of environmental protozoan (oo)cysts to UV are appropriate.

Data from the evaluated studies indicated that photo-reactivation can result in a significant increase of the required fluence for bacteria to achieve the same level of inactivation as without photo-reactivation. Dark repair does not seem to be very significant for the UV disinfection practice for most pathogens. Though for *Giardia*, dark repair was observed in two studies at lower (5–25 mJ/cm^2) fluences, but not at higher fluences (60 mJ/cm^2). Consequently, correction of the required fluence of full-scale UV disinfection because of photo-reactivation of bacteria and in the case of *Giardia*, also because of dark repair at low fluences, could be necessary. For viruses, it is assumed that repair is included in the available fluence–response curves, as suggested for the double-stranded DNA Adenoviruses. After UV disinfection of drinking water, photo-reactivation is not likely to occur but in the case of wastewater disinfection light exposure is likely. Translation of the presented photo-reactivation data to full-scale conditions, however, is not straight forward. These data have been observed under conditions favouring the induction of photo-repair (low fluence values, thin layer with optimal conditions for reactivation). The conditions in wastewater practice will be less favourable for exposure to light and hence for photo-reactivation to occur. Furthermore, the applied UV fluences in practice are usually higher than applied in the reviewed studies. Lindenauer and Darby (1994) and Gehr and Nicell (1996) showed a decrease in repair at higher fluences due to tailing in the inactivation kinetics. The former author also hypothesised that extended DNA damage at higher fluence values will reduce the potential for photo-repair. From their WWS and that of Whitby and Palmateer (1993), Gehr and Nicell (1996) suggested that in practice the overall impact of photo-repair might be negligible, because of the limited exposure to light and therefore limited induction of photo-repair. In conclusion this needs further verification, but we assume that the necessity for a fluence correction as a result of photo-reactivation in UV disinfection practice is less than that suggested by the experimental data in the photo-reactivation studies.

4.4. *Correction of the required fluence: fluence-related factors*

Most of the studies that have been reviewed have been executed under well-controlled laboratory conditions in which UV fluence was assessed with sensors and seeded micro-organisms. Information about the efficacy of UV systems under full-scale conditions was limited and those which have been evaluated, generally showed lower inactivation efficiency than in the laboratory. This reduced efficiency may be caused by factors related to the micro-organisms as described previously, but also by imperfections in the calculation of the fluence to which the micro-organisms are exposed in full-scale UV systems. The latter can be largely overcome by applying biodosimetry to full-scale UV systems to determine the REF. This is already enforced for the application of UV systems in drinking water practice in Austria (Österreichisches normungsinstitut, 1999). In Germany, a similar protocol is used as guideline (DVGW, 1997), and in the USA, the draft EPA Ultraviolet Disinfection Guidance Manual also appoints credits for inactivation of *Cryptosporidium* on the basis of biodosimetry (USEPA, 2003). Commonly used biodosimeters are spores of *B. subtilis* or MS2 phages. Cabaj et al. (1996), however, demonstrated that the REF decreases with increased broadening of the fluence distribution and increased inactivation rate constant of the used model organism. Consequently, susceptible model organisms (high *k*-value) are more sensitive to a broad fluence distribution, which will enlarge the gap between the REF and the arithmetic mean fluence. MS2 phages and spores of *B. subtilis* are less sensitive to UV than most other pathogenic micro-organisms (Tables 1–4). The EPA manual (USEPA, 2003) introduced an REF bias based on effects of fluence distribution and inactivation rate constants to account for the difference in sensitivity between model organism and target pathogens. Another approach is the use of alternative model organisms. *E. coli* is suggested and also this review indicates that it can be used as model for the more susceptible bacteria and also *Cryptosporidium* and *Giardia*. More recently, Clancy et al. (2004) suggested two potential bacteriophages Qβ and T7 as model organisms. The use of T7 as UV dosimeter was previously proposed by Rontó et al. (1992). The *k*-values of these organisms (Table 2) are more in the range of the *k*-values calculated for the more sensitive pathogens. For MP systems, the germicidal fluence is usually obtained with the DNA absorbance spectrum to weigh the effectiveness of the different

Table 6 – The UV fluence (mJ/cm^2) requirements for an MIC of 1 up to 4 log by monochromatic UV radiation for viruses, bacteria, bacterial spores and protozoan (oo)-cysts based on the k-values with or without correction for environmental species; for bacteria in wastewater a higher correction for environmental species is needed and further research has to clarify the need for a higher fluence to account for photoreactivation; for Giardia increased fluence requirement because of dark repair is a factor for further research

	Required fluence (mJ/cm^2)			
MIC required (log):	1	2	3	4
Bacillus subtilis[a]	56	111	167	222
Adenovirus type 40	56	111	167	—[b]
Clostridium perfringens[a]	45	95	145	—[b]
Adenovirus type 2, 15, 40, 41	42	83	125	167
Acanthamoeba[c]	40	71	119	167
Adenovirus[a] (no type 40)	25	50	—[b]	—[b]
Calicivirus canine	10	21	31	41
Rotavirus SA-11	10	20	29	39
Calicivirus feline	9	19	28	38
Coxsackie virus B5	8	17	25	34
Streptococcus faecalis[a]	9	16	23	30
Legionella pneumophila[d]	8	15	23	30
Poliovirus type 1	7	15	22	30
Shigella sonnei[d]	6	13	19	26
Salmonella typhi[a]	6	12	17	51
Hepatitis A	6	11	17	22
Calicivirus bovine	5	11	16	21
E. coli O157[d]	5	9	14	19
E. coli[a]	5	9	14	18
Cryptosporidium USEPA[c]	3	6	12	—[e]
Giardia USEPA[c]	2	5	11	—[e]
Campylobacter jejuni[d]	3	7	10	14
Yersinia enterocolitica[d]	3	7	10	13
Legionella pneumophila[d]	3	6	8	11
Shigella dysenteriae[d]	3	5	8	11
Vibrio cholerae[d]	2	4	7	9

[a] Environmental spp.
[b] $MIC_{max} < 4$ log.
[c] No correction for environmental spp. (research needed).
[d] corrected for environmental spp.
[e] No value due to tailing.

wavelengths. The action spectra (the relative sensitivity to different UV wavelengths) of adenoviruses and the model organism MS2 phage differed from the action spectrum of DNA as demonstrated by Malley et al. (2004) and Mamane-Gravetz et al. (2005). Thus, with different action spectra of model organisms used as biodosimeter different REF values will be calculated for PC UV radiation. Therefore, more information about differences in action spectra between pathogens and potential biodosimeter organisms is required to increase the precision of the fluence determination of MP systems.

4.5. *Required fluence table*

The accumulated knowledge in this review was used to create the required fluence of LP lamps for a MIC of 1, 2, 3 or 4 log inactivation for most micro-organisms relevant to microbiological safety of water (Table 6). The required fluence is calculated from the k-values presented in Tables 1, 3 and 4 with correction for the increased UV sensitivity of environmental bacteria and bacterial spores. For five bacteria species, this was based on specific literature data and for the other bacteria, this was set at a factor of three, whereas for wastewater disinfection higher correction seems appropriate. Correction for environmental organisms was not necessary for the viruses (see data on FRNA phages in Fig. 7) and for the protozoa no data are available. Increased fluence requirement because of DNA repair did not seem necessary for viruses and protozoa, although for *Giardia* at low fluences of 5–25 mJ/cm^2 results are still conflicting. For bacteria, fluence correction for dark repair is not necessary and further study has to elucidate whether correction for photo-reactivation is required.

4.6. *Research items*

Based on this review a number of knowledge gaps are identified. More quantitative information is needed to estimate the effect of micro-organism-related factors like environmental species, DNA repair (esp. of *Giardia*) and differences in spectral sensitivity influencing the fluence requirement of UV disinfection under full-scale conditions. Biodosimetry is a powerful tool to determine germicidal fluence values of CF systems, but to determine REF for the whole range of relevant micro-organisms with different UV sensitivities, additional model organisms are needed. In the application of medium-pressure lamps with PC UV light, further development of fluence assessment is of importance using biodosimetry with proper weighting for spectral sensitivity in connection with fluence calculation models. Daily UV process control needs further research into accurate description of the distribution of water flows and UV intensity over UV reactors, using CFD, to obtain simple, reliable and cheap in situ process control systems. Independent verification with biodosimetry is still essential. Systems to measure the germicidal fluence on-site in water treatment practice, using micro-organisms or compounds "naturally" present in the water would allow on-site verification of the efficacy of UV systems in practice.

5. Conclusions

The accumulated literature data on the inactivation kinetics of disinfection with UV irradiation demonstrate that the process is effective against all pathogenic micro-organisms relevant for the current drinking water practices. The inactivation of micro-organisms by UV could be described with first-order kinetics using fluence-inactivation data from laboratory studies in collimated beam tests. No inactivation at low fluences (shoulder) and no further increase of inactivation at higher fluences (tailing) was observed for some micro-organisms. The former deviation from the log-linear kinetics is included in MIC calculations and the latter was used to determine the maximum (observed) MIC values. The parameters that were used to describe the inactivation are the inactivation rate constant k (cm^2/mJ),

the maximum inactivation demonstrated and (only for bacterial spores and *Acanthamoeba*) the offset parameter. The most persistent organisms known are viruses, specifically Adenoviruses, and bacterial spores. From the protozoa *Acanthamoeba* was highly UV resistant. Bacteria and (oo)cysts of *Cryptosporidium* and *Giardia* are more susceptible with a fluence requirement of $<20\,mJ/cm^2$ for an MIC of 3 log.

Several studies have reported an increased UV resistance of environmental bacteria and bacterial spores, compared to lab-cultured organisms. This means that higher UV fluences are required to obtain inactivation. Hence, for bacteria and spores, a correction factor of two and four was included in the MIC calculation, respectively, and data from the WWS show that a higher correction is required under these conditions. For phages and viruses this phenomenon appears to be of little significance and for protozoan (oo)cysts this aspect needs further attention. For application in drinking water, no correction for repair seems necessary for most pathogens. The results on repair for *Giardia* are conflicting, but no repair occurred at higher fluences ($60\,mJ/cm^2$). For application in wastewater, the occurrence of photo-reactivation of bacteria is a subject for further research. To enable accurate assessment of the effective fluence in CF UV systems in water treatment practice, biodosimetry is of great importance. In the case of MP lamps, more information about differences in spectral sensitivity between pathogens and potential biodosimeter organisms is needed to increase the precision of the fluence determination. For UV systems that are primarily dedicated to inactivate the more sensitive pathogens (*Cryptosporidium, Giardia*, pathogenic bacteria), additional model organisms are needed to serve as biodosimeter.

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