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The Effect of UV and Combined Chlorine/UV Treatment on Coliphages in Drinking Water Disinfection

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Abstract: Ultraviolet (UV) irradiation is a common way to disinfect drinking water, but some viruses are very resistant to UV. Drinking water was disinfected with UV after spiking with MS2 and 18 different coliphages isolated from municipal wastewater effluent. In addition, some coliphages were disinfected with combined treatment of chlorine/UV or *vice versa* with UV/chlorine. A UV-dose of 22 mWs/cm^2 caused less than 2 Log_{10}-reductions of 10 UV-resistant strains, while it caused up to 7 Log_{10}-reductions for 9 UV-sensitive or intermediate strains. The high dose (117 mWs/cm^2) caused only 3 Log_{10}-reductions in some UV-resistant coliphages, including MS2, which proved to be a good indicator for viruses in UV-disinfection tests. The combined treatment with 0.1 or 0.5 mg Cl/L (free Cl-dosage 0.04 or 0.2 mg/L, respectively) for 10 min followed by UV irradiation of 22 mWs/cm^2 inactivated all coliphages tested by >3.6 Log_{10}-units. Synergy was obtained for most coliphages tested by using a Cl/UV combination, and the inactivation using first low Cl-dosages followed by low UV-dosages was higher than if using high Cl- or UV-dosages alone. The opposite treatment with UV/Cl was less effective. Therefore, the combination treatment using first chlorine and then UV can be recommended as a disinfection method for viruses.

Keywords: coliphages; ultraviolet irradiation; chlorination; combination; disinfection; water

1. Introduction

Drinking water safety is often jeopardized by the presence of disease-causing microorganisms, such as human viral pathogens [1]. Many viruses originate from human and animal feces, which contaminate drinking water sources due to, for example, poor sanitation [2], floods and surface runoffs [3], or malfunction of wastewater treatment systems.

Drinking water treatment plants reduce high numbers of pathogens by conventional pre-treatments including coagulation, sedimentation, and filtration processes, but disinfection must be applied to inactivate pathogens and guarantee the safety of drinking water [4]. UV is widely applied for controlling microbial contamination in drinking water, wastewater, and different industrial waters [5,6]. The UV may partly destroy microorganisms without compromising the taste or odor of water and without forming disinfection by-products associated with chlorination [7]. In addition, UV-treatment needs only a short contact time, leading to minimal space requirement, and it does not cause corrosion in the water distribution system. However, some microorganisms, especially viruses, have a high resistance against UV irradiation [8,9]. Another disadvantage is that UV cannot guarantee safe drinking water if the distribution system is contaminated with even a low number of surviving

microorganisms, because UV irradiation does not provide the residual disinfection effect of chemical disinfectants [10].

Practical application of UV disinfection relies on the germicidal ability of UVC and UVB irradiation (λ = 200–260 nm), which damages nucleic acids of microorganisms by absorption of nucleotides, the building blocks of RNA and DNA [11]. Viruses have no repair mechanisms to reverse the damage created by UV irradiation, but may use the repair enzymes of their host cells [12,13].

There is also variation in the UV-resistance between different viruses. For example, hepatitis A virus requires a UV-dose of only 0.184 mWs/cm^2 to achieve 4 Log$_{10}$-reduction [14]. Resistant viruses, such as adenovirus and MS2-bacteriophage, achieve 2–4 Log$_{10}$-reductions at UV-doses between 48–226 mWs/cm^2 [9,15,16]. MS2 is often used as an indicator for viruses (*i.e.*, a surrogate virus) in drinking water [17], since its size and structure are similar to many enteric viruses and it is easy to quantitatively analyze in the laboratory.

UV irradiation may be combined with chemical compounds to achieve better disinfection efficiency than if either one is used alone. Drinking water treatment plants often combine UV and chlorine sequentially, so that there is first UV and then chlorination. This combination treatment has shown high inactivation for viruses in laboratory experiments [18,19]; for example 4 Log$_{10}$-reductions of adenoviruses have been achieved with a UV-dose of 10 mWs/cm^2 followed by 0.17 mg free Cl/L within a contact time of only 1.5 min [19]. However, this combination has given controversial results on synergy [15,16,18], *i.e.*, the inactivation of viruses with the combined treatment has not always been higher than the sum of inactivations obtained by single treatments.

On the contrary, high synergy has been observed against viruses by using UV and chemical disinfection in the opposite order, in either simultaneous or sequential processes, when the chemicals were not quenched [15,16,18,20]. For example, combining treatment with 0.15 mg free Cl/L and UV-dose of 50 mWs/cm^2 gave 4 Log$_{10}$-reductions for adenoviruses, which was higher than the sum of reductions obtained with Cl- or UV-treatment alone [16]. This order of combination is not very common, because UV irradiation degrades chlorine and may reduce the amount of residual chlorine in distribution systems. Nevertheless, the combination order of Cl followed by UV might have potential for disinfection and should be studied more.

The main aim of this study was to find new disinfection methods against viruses in drinking water. Thus, we studied the susceptibility of MS2 and 18 coliphage strains on different UV doses and combined treatments using low Cl-dose with a short contact time and low UV-dose. Further, we compared the efficiencies of combinations Cl/UV and UV/Cl on disinfection to find out possible synergies of these treatments.

2. Materials and Methods

2.1. The Origin of Coliphages

The coliphages were isolated from wastewater effluent as described before [21] by using a double-layer technique [22] for the cultivations and determinations of phage density. The isolated coliphages and MS2 (strain ATTC 15597-B1) were enriched as described earlier [23,24]. The host bacteria were *Escherichia coli* ATCC 13706 and *E. coli* ATCC 15597. The concentrations of coliphages in stock solutions were approximately 10^9 PFU/mL.

2.2. UV Experiments

UV disinfection was carried out with a collimator device in which a low-pressure mercury arc lamp (Osram HNS 30 W, λ = 253.7 nm, Munich, Germany) was used as the source of UV irradiation. The UV lamp was turned on for at least 15 min before initiation of the experiment to obtain a constant UV intensity output.

Ten milliliters of coliphage stock solution was pipetted to a sterile glass Petri dish (inner diameter 6.0 cm), so that the UV irradiation beam was directly focused onto the Petri dish via the collimator

tube. The solution was magnetically stirred throughout the UV irradiation experiment, and the solution was exposed to UV-doses between 22–117 mWs/cm^2 by using different exposure times. The average UV-doses were determined as the product of the UV intensity and the exposure time in seconds [25]. The intensity on the sample surface in the Petri dish measured by OL 756 Portable High-Accuracy UV-Visible Spectroradiometer (Optronic Laboratories Inc., Orlando, FL, USA) was approximately 0.2 mW/cm^2. After the UV disinfection, a 1-mL sample was taken for the determination of coliphage densities, as described in Section 2.1. Transmittance of the water was 87%, calculated from the absorbance of the water measured with a spectrophotometer (UV-2401PC, Shimadzu, Kyoto, Japan) at a wavelength of 254 nm. The tests were carried out in three parallels at a room temperature of 20 °C–21 °C and a pH of 7.2–7.4.

2.3. Combined Cl and UV Disinfection Tests

The tests were carried out using Kuopio tap water, which was dechlorinated overnight. The annual means of water in the years 2013–2015 were for turbidity 0.10–0.11 FTU, chemical oxygen demand (COD_{Mn}) 1.3–1.4 mg/L, color < 5 mg Pt/L, total organic carbon (TOC) 2.1 mg C/L, and numbers of *E. coli* and enterococci 0 CFU/100 mL according to the data of Kuopion Vesi [26]. The isolated coliphages and MS2 were first exposed to total chlorine concentrations of 0.1 or 0.5 mg/L (free Cl-dosage 0.04 or 0.2 mg/L, respectively) with contact times of 3 to 10 min, which resulted in Ct values of approximately 0.1 and 2 mg free chlorine × min/L, respectively. Then, the disinfection treatment was continued with UV treatment with dose of 22 mWs/cm^2 without quenching the residual Cl before starting UV treatment.

The other combination test was done for selected coliphage strains by using first UV-dose of 22 mWs/cm^2 and then immediately adding 0.1 or 0.5 mg Cl/L (free Cl-dosage of 0.04 or 0.2 mg/L, respectively) up to 10 min of contact time. The coliphages densities were analyzed as described above in 2.1 without quenching the reaction before the cultivation.

2.4. Calculations and Statistical Analyses

Inactivation values, *i.e.*, logarithmic reductions, were calculated as the Log_{10} (N/N_0), where N is the coliphage density after the treatment and N_0 the density before the experiment. The detection limit for the density of coliphages was 10 PFU/mL. If no plaques were found on dishes, half of it, *i.e.*, 5 PFU/mL, was used for the calculations. Related sample Friedman's two-way analysis, with SPSS version 22, was used to determine if UV-disinfection had a statistically significant effect on coliphage density. Differences were considered significant at $p < 0.05$ compared to the control (without UV-disinfection). Linear regression equations for the means of all three parallel UV treatments were calculated by the least square method with Excel 2013 to describe the relationship between Log_{10}-reduction and UV-dose. If the detection limit was reached in all three parallels and the Log_{10}-reduction was $\geqslant$ the maximum reduction, this dose point was not used for calculating the linear regression line. To find out the statistically significant differences between coliphage strains against UV-disinfection, the slopes of three separate parallel linear regression equations for each strain were analyzed by a non-parametric Kruskall-Wallis test ($p < 0.05$) (SPSS 22).

Synergy values were counted according to equation [20]:

Synergy as Log_{10}-units = Log_{10}-reduction of combined chemical/UV disinfection − (the Log_{10}-reduction for UV disinfection + the Log_{10}-reduction by chemical disinfection).

The positive value of synergy means a synergistic effect. The negative value means antagonistic effect and zero value means the efficiency of combined treatment was the same as the sum of the two individual treatments.

3. Results

3.1. Inactivation of Coliphages by UV

The coliphages were divided into 10 UV-resistant (including MS2) and 9 UV-sensitive or intermediate coliphages according to statistically significant differences between the slopes of the regression equations ($p < 0.05$) (Tables 1 and 2).

A UV-dose of 22 mWs/cm^2 caused less than 2 Log_{10}-reductions of the UV-resistant strains, and as high a dose as 117 mWs/cm^2 caused only 3 Log_{10}-reductions in coliphages strain 14 and MS2 (Table 1). The inactivation of resistant coliphages by UV was linear with slopes between −0.02 and −0.07 at UV-doses from 0 to 117 mWs/cm^2, with high values of coefficient of determination (R^2-values) in their linear regression equations.

Table 1. Log_{10}-densities (Mean ± SD, n = 3) and (Log_{10}-reductions in parenthesis) of MS2 and the isolated 18 coliphages before and after different doses of UV (mWs/cm^2). Statistically significant differences from the control (UV dose 0 mWs/cm^2), assessed by related sample Friedman's two-way analysis of variance, are indicated with asterisks, * $p < 0.05$. LDL = less than the detection limit (<10 PFU/mL) in all three parallels, ldl = less than the detection limit in one or two parallels.

Coliphages	Log_{10}-Densities at UV: 0 mWs/cm^2	Log_{10}-Densities and (Log_{10}-Reductions in Parenthesis) after Different UV-Treatments				Linear Regression Equations and Their R^2-Values: y = Log_{10}-Reduction x = UV-Dose (mWs/cm^2)
		UV: 22 mWs/cm^2	UV: 47 mWs/cm^2	UV: 82 mWs/cm^2	UV: 117 mWs/cm^2	
14	8.49 ± 0.02	8.43 ± 0.04 (0.06)	7.49 ± 0.05 (0.99)	6.48 ± 0.03 (2.01)	5.46 ± 0.01 * (3.03)	y = −0.02x (R^2 = 0.83)
MS2	8.09 ± 0.04	8.00 ± 0.04 (0.09)	6.97 ± 0.04 (1.12)	5.86 ± 0.02 (2.23)	4.75 ± 0.03 * (3.35)	y = −0.03x (R^2 = 0.96)
5	7.66 ± 0.09	6.99 ± 0.10 (0.67)	6.14 ± 0.04 (1.52)	4.03 ± 0.20 * (3.63)	2.32 ± 0.08 * (5.35)	y = −0.04x (R^2 = 0.98)
3	9.64 ± 0.13	8.14 ± 0.07 (1.51)	6.67 ± 0.09 (2.97)	4.76 ± 0.09 * (4.88)	3.53 ± 0.21 * (6.11)	y = −0.06x (R^2 = 0.98)
18	8.84 ± 0.08	7.61 ± 0.44 (1.23)	6.09 ± 0.07 (2.76)	4.06 ± 0.03 * (4.78)	2.56 ± 0.14 * (6.28)	y = −0.06x (R^2 = 0.99)
13	8.36 ± 0.02	6.83 ± 0.16 (1.53)	5.33 ± 0.05 (3.03)	3.40 ± 0.01 * (4.96)	LDL *	y = −0.06x (R^2 = 0.99)
17	5.51 ± 0.24	3.86 ± 0.04 (1.66)	2.65 ± 0.18 (2.86)	1.11 ± 0.56 ldl * (4.40)	LDL *	y = −0.06x (R^2 = 0.97)
9	6.75 ± 0.05	4.61 ± 0.05 (2.14)	3.69 ± 0.15 (3.06)	1.36 ± 0.17 * (5.39)	LDL *	y = −0.06x (R^2 = 0.90)
1	7.43 ± 0.06	6.19 ± 0.10 (1.24)	4.36 ± 0.46 (3.08)	1.82 ± 0.17 * (5.62)	LDL *	y = −0.06x (R^2 = 0.96)
6	8.17 ± 0.02	7.06 ± 0.11 (1.11)	4.03 ± 0.11 (4.14)	1.76 ± 0.22 * (6.41)	LDL *	y = −0.07x (R^2 = 0.94)
16	9.22 ± 0.04	7.65 ± 0.08 (1.58)	4.92 ± 0.25 (4.31)	1.71 ± 0.67 * (7.52)	LDL *	y = −0.08x (R^2 = 0.93)
15	9.84 ± 0.04	7.68 ± 0.22 (2.15)	4.14 ± 0.05 (5.70)	1.83 ± 0.09 * (8.01)	LDL *	y = −0.09x (R^2 = 0.91)
4	9.69 ± 0.05	3.25 ± 0.17 (6.44)	2.44 ± 0.05 * (7.25)	2.28 ± 0.08 * (7.41)	LDL *	y = −0.09x (R^2 = 0.32)
12	8.32 ± 0.03	4.45 ± 0.21 (3.87)	2.33 ± 0.13 (5.99)	1.11 ± 0.41 ldl * (7.21)	LDL *	y = −0.10x (R^2 = 0.82)
7	8.17 ± 0.07	4.09 ± 0.03 (4.08)	2.46 ± 0.20 (5.71)	LDL *	LDL *	y = −0.10x (R^2 = 0.81)
11	8.34 ± 0.13	4.70 ± 0.09 (3.64)	2.22 ± 0.03 (6.13)	1.15 ± 0.85 ldl * (7.20)	LDL *	y = −0.11x (R^2 = 0.88)
10	8.57 ± 0.02	2.54 ± 0.24 (6.03)	2.01 ± 0.10 * (6.55)	LDL *	LDL *	y = −0.11x (R^2 = 0.52)
8	8.42 ± 0.01	4.20 ± 0.14 (6.03)	1.51 ± 0.49 * (6.92)	LDL *	LDL *	y = −0.12x (R^2 = 0.75)
2	10.1 ± 0.14	2.73 ± 0.26 * (7.33)	2.18 ± 0.20 * (7.88)	LDL *	LDL *	y = −0.14x (R^2 = 0.51)

The most UV-sensitive strain (2) was inactivated up to 7 Log_{10}-units already with the dose of 22 mWs/cm^2, and all UV-sensitive and intermediate strains were inactivated more than 4 Log_{10}-units with the dose of 47 mWs/cm^2 (Table 1). The slopes were less than −0.08 for UV-sensitive or intermediate coliphages. The slopes of the UV-sensitive strains were statistically different from the slopes of the UV-resistant strains, while the slopes of the intermediate strains did not differ from those of UV-resistant or UV-sensitive strains (Table 2).

Table 2. Grouping of coliphage strains to UV-resistant, -intermediate and -sensitive based on statistically significant differences between the slopes of linear regression equations (k = Log_{10}-reduction/UV dose) of the strains (* $p < 0.05$, Kruskall-Wallis test). The table shows only the statistically significant *p*-values.

Coliphages		Intermediate			Sensitive					
		16	**15**	**4**	**12**	**7**	**11**	**8**	**10**	**2**
Resistant	**14**				0.002 *	0.002 *	0.002 *	0.000 *	0.000 *	0.000 *
	MS2				0.005 *	0.004 *	0.003 *	0.001 *	0.000 *	0.000 *
	5				0.009 *	0.008 *	0.003 *	0.002 *	0.001 *	0.000 *
	3				0.009 *	0.035 *	0.028 *	0.010 *	0.005 *	0.003 *
	18				0.027 *	0.025 *	0.019 *	0.007 *	0.004 *	0.002 *
	13							0.029 *	0.017 *	0.009 *
	17							0.021 *	0.013 *	0.006 *
	9							0.020 *	0.012 *	0.006 *
	1								0.035 *	0.019 *
	6									0.046 *

The R^2-values of linear regression equations were low for many sensitive or intermediate coliphages, indicating a tailing effect. Thus, the regression line was no more linear when the number of coliphage plaques was low (Table 1). The R^2-values were low in spite of the fact that we omitted the results where the Log_{10}-reductions exceeded the detection limits at the highest UV-doses when calculating the regression equations.

3.2. Inactivation of Coliphages with the Combined Cl/UV and UV/Cl Treatments

High inactivation of UV-resistant coliphage strains 14, 5, 17, 1, and 6 (Tables 1 and 2), which were previously tested to be also chlorine-resistant [20], and MS2 was achieved when disinfection was done in a combination treatment using first Cl and then UV (Table 3). Log_{10}-reductions of 2.5– >5.4 were achieved for these coliphages, if the total chlorine dose was 0.1 mg/L (free Cl-dosage of 0.04 mg/L) with contact times of 3–10 min before UV irradiation with 22 mWs/cm^2 (Table 3). In the combination treatment 10 min of contact time of chlorine showed very good efficiency. Strains 18 and 4, previously found to be sensitive to chlorine [21], were also high, 8.7 and >10.7 Log_{10}-reductions, respectively.

Table 3. The Log_{10}-reductions of coliphages during disinfection with Cl alone, UV alone or combined Cl and UV treatment (Mean ± SD, $n = 3$).

Coliphages	Log_{10}-Reductions in Disinfection Treatment				
	Cl Alone for 10 min		**UV Alone 22 mWs/cm^2**	**Combined Treatment with Cl 0.1 mg/L (0.04 mg free/L) and UV 22 mWs/cm^2**	
	0.1 mg/L (0.04 mg free/L)	**0.5 mg/L (0.2 mg free/L)**		**Cl-treatment time (min)**	**Reduction**
14	0.15 ± 0.13	0.59 ± 0.26	0.06 ± 0.04	10	4.52 ± 0 [a]
MS2	1.70 ± 0.04	>6.06 ± 0 [b]	0.09 ± 0.04	10	3.63 ± 0.35
				10	>5.43 ± 0 [b]
5	0.12 ± 0.10	0.02 ± 0.13	0.67 ± 0.10	7	2.66 ± 0.10
				3	2.52 ± 0.19
18	6.27 ± 0.01	7.04 ± 0.02	1.23 ± 0.44	10	8.73 ± 0.19
17	0.26 ± 0.19	0.50 ± 0.06	1.66 ± 0.04	7	3.91 ± 0.02
1	0.35 ± 0.04	0.03 ± 0.15	1.24 ± 0.10	10	>5.19 ± 0 [b]
				10	5.12 ± 0.50
6	0 ± 0.07	0.24 ± 0.25	1.11 ± 0.11	7	4.03 ± 0.13
				3	2.89 ± 0.02
4	2.02 ± 0.21	5.36 ± 0 [b]	6.44 ± 0.17	10	>10.7 ± 0 [b]
7	0.21 ± 0.01	0.07 ± 0.11	4.08 ± 0.03	7	3.79 ± 0.05

[a] 0.5 mg Cl/L, [b] Detection limit reached.

The calculation of synergy values was based on Log_{10}-reductions shown in Table 3. For example, for coliphage 14, the synergy is 4.52 − (0.59 + 0.06) = 3.87 when using a chlorine concentration of 0.5 mg/L (Tables 3 and 4). In many cases, the exact synergistic effect could not be calculated since the detection limit of coliphages was reached (Table 3).

Table 4. Synergy values of coliphages when chlorine treatment with 0.1 or 0.5 mg/L was done first followed by UV of 22 mWs/cm^2 or UV treatment was done first followed by chlorine with 10 min contact time.

Coliphages	CL Dose mg/L	Chlorine Treatment First and then UV Dose	UV Treatment First and then Chlorine
14	0.5	3.87	2.17
MS2	0.1	1.84	0.29
18	0.1	1.23	0.72
4	0.1	2.27	1.50
17	0.1	1.99	nt
1	0.1	>3.60 [a]	nt
7	0.1	–0.50	nt

[a] Detection limit was reached in the combination test, nt = not tested.

Eight of nine coliphages tested including MS2 showed synergy in chlorine/UV combination (Tables 4 and 5). An increasing chlorination time in the combination treatment led to increased synergy for the tested coliphages 5 and 6 (Table 5). The only coliphage that did not show any synergy was strain 7 (Table 4). This strain was very chlorine-resistant but UV-sensitive and only seven mines of contact time with chlorine was tested (Table 3), which may explain the lack of synergy. Clearly higher synergy values were achieved by combining first Cl and then UV disinfection instead of using first UV and then chlorine (Table 4).

Table 5. Synergy values of coliphages when first treated with 0.1 mg/L chlorine for different times and then UV doses of 22 mWs/cm^2.

Coliphages	Chlorine Treatment Times			
	3 min	5 min	7 min	10 min
5	1.73	1.85	1.87	>4.64 [a]
6	1.78	nt	2.92	4.01

[a] Detection limit was reached in the combination test, nt = not tested.

4. Discussion

Our study confirmed that MS2 is a good indicator virus for UV disinfection, since it was very UV-resistant, even at the highest UV-dose tested (117 mWs/cm^2). The typical UV-dose required for 4 Log_{10}-inactivation of MS2 has been 85 mWs/cm^2 [17]. Some studies report that UV-doses between 34 and 119 mWs/cm^2 inactivated 2 to 4 Log_{10}-units of MS2 [9,13,27]. Many studies have shown that MS2 is more resistant against UV than many other viruses, such as poliovirus type 1 [28], coliphages T4 and T7 [29], hepatitis A virus [14], and feline calicivirus [9] but less resistant than adenoviruses 40 and 41 [6,9]. Some adenoviruses may need up to 201 mWs/cm^2 for 3 Log_{10}-reductions [17]. Thus, our results (Table 1) and the studies referred to confirm that much higher doses than the 40 mWs/cm^2 recommended by the NSF/ANSI [30] are needed for the inactivation of many viruses.

Our most important finding was that UV-resistant coliphages could be inactivated in combination treatment when using chlorine without quenching, followed by UV irradiation. There was thus a high synergistic inactivation for most of the tested coliphages. The synergistic effect of chlorine/UV could appear when disinfection started with 0.1 mg/L with 10 min of contact time (Ct 0.4 mg free chlorine× min/L) and continued with 22 mWs/cm^2 UV irradiation. A chlorine contact time of 3 min

already had a synergistic effect, but longer contact times, such as 10 min, were more effective—the detection limit was often reached. Possibly longer times could be still more beneficial, especially if the quality of the water is poor, and this should be studied more. Very similarly to our study, the exposure to free Cl-doses of 1 mg/L or 1.5 mg/L (Ct value of 0.41 mg free chlorine× min/L) followed instantly by UV-doses of 17 or 51 mWs/cm^2 caused 2–6 Log_{10}-reductions of MS2 [18]. Up to 4 Log_{10}-reductions has been achieved for adenovirus using only 0.15 mg/L free chlorine doses combined with UV doses 50 mWs/cm^2 [16]. Thus, the combined effect of chlorine/UV is more effective than either UV or chlorine treatments alone [15,16,18], and if treatment is sequential instead of simultaneous [16]. When the combined disinfection was done in the present work using first a UV-dose of 22 mWs/cm^2 and then chlorination with 0.1 mg/L total Cl/L for 10 min, there was lower or almost no synergy (Table 4), confirming an earlier result [16]. This also suggests that the combined order of chlorine/UV is better than UV/chlorine, and high inactivation of viruses can be obtained with chlorine and UV dosages used nowadays in drinking water treatment plants.

Chlorine causes damage to the surface structures of coliphages by breaking the chemical bonds in proteins and enzymes [31]. The UV irradiation targets the nucleic acids [32]. It is also possible that the radicals formed during the combined effect of chlorine and UV irradiation [33] were responsible for damage in virus particles. This is supported by the inactivation results of UV/chlorine combination, which gave clearly lower synergism effects than chlorine/UV treatment. Thus, the combined application of Cl and UV disinfection methods may allow use of lower chlorine dosages or less electricity for UV than the opposite way UV/chlorine. In water disinfection, this combined treatment could save money.

In our work, when determining linear regression lines between the coliphage reductions and UV-doses, a few coliphages were still detected at relatively high UV-doses. The coefficients of determination (R^2) were low in these cases. It may be that this tailing effect of coliphages can be caused by a clumping of virus particles with impurities of water and with each other, and viruses in these clumps may be protected against disinfection [8]. Viruses may also attach to the walls of the disinfection vessel so that UV cannot penetrate to all virus particles making their destruction difficult. If this phenomenon is found, the disinfection doses and times must be increased.

Here, we have analyzed the effect of UV on tested coliphages in a collimator device where the UV penetration is good and in water with a turbidity of only 0.10–0.11 FTU and a color less than 5 mg Pt/L [26]. Water treatment before disinfection is thus important for reaching a high quality of water to guarantee the efficiency of disinfection. If the water to be disinfected had more color or turbidity, there would be a higher need of chlorine and/or UV irradiation and possibly additional pre-treatments [4]. The work should be continued using water with lower quality than was used by us, which is a reality for many parts of the world. The combined chlorine/UV disinfection seems to be a better choice for a water treatment plant than using first UV, followed by chlorine or using higher doses of either chlorine or UV alone. The necessary doses of chlorine and UV must be studied in each water plant separately. Post-chlorination may be needed to protect the distribution pipe system against resistant organisms, such as different viruses, *Ascaris* eggs, *Rubrobacter radiotolerans*, *Deinococcus* spp., and endospores of *Bacillus* spp.

5. Conclusions

In conclusion, a high variation in the sensitivity of different coliphage strains to UV was noticed. Higher doses than the recommended 40 mWs/cm^2 [17] was needed to destroy the most resistant coliphages. MS2 was very UV-resistant and proved to be a good indicator for UV disinfection. In contrast to individual chlorine or UV-treatments, a combined treatment, with a low dosage of chlorine followed by low dosages of UV, showed high synergy values and efficiently inactivated the UV- and Cl-resistant coliphages. Synergy values were lower if the order of the combined treatment was reversed. Thus, the combination treatment with first chlorine followed by UV can be recommended for disinfection of viruses.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

UV	ultraviolet
Cl	chlorine
COD_{Mn}	chemical oxygen demand
TOC	total organic carbon

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