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Comparison of UV and chlorine inactivation of particle and non-particle associated coliform

B. Örmeci and K.G. Linden

Department of Civil and Environmental Engineering, Box 90287, Duke University, Durham NC 27708, USA

Abstract Bacteria associated with particles may be sheltered from chlorine and ultraviolet (UV) disinfection. The objective of this study was to compare the disinfection effectiveness of UV irradiation and free chlorine for naturally occurring particle-associated coliform (PAC) and non-particle associated coliform (NPAC) in wastewater using a single wastewater source, under identical laboratory protocols. Samples containing NPAC were obtained using three different methods: EGTA extraction, filtration, and blending followed by filtration. Unaltered wastewater was used for samples containing PAC. Wastewater samples were inactivated with UV irradiation at doses between 0–100 mJ/cm^2, and with chlorine at CT values between 0–525 mg min/L. The dose-survival relationships and inactivation rates between PAC, NPAC, and chlorine and UV irradiation were then compared. As expected, both UV and chlorine were effective for inactivation of NPAC. However, under prolonged contact times, chlorine appears to be more effective for inactivation of PAC than UV irradiation. Contact time appears to be the most important factor in determining the effectiveness of chlorine disinfection of PAC, and chlorine CT alone was not a good indicator of disinfection effectiveness in wastewater. PAC was found to survive at UV and chlorine disinfection doses typically applied in a wastewater treatment plant.

Keywords Chlorine; coliform; disinfection; particle-associated; ultraviolet; wastewater

Introduction

Chlorination and ultraviolet (UV) irradiation are two widely used methods of disinfection for wastewater. Specific advantages and disadvantages for each process in wastewater treatment have been described previously in the literature (e.g., USEPA, 1986; Darby *et al*., 1995). It has been known that microorganisms that are attached to surfaces or associated with particles may be protected from chlorine and UV disinfection (LeChevallier *et al*., 1984; Herson *et al*., 1987; Berman *et al*., 1988; LeChevallier *et al*., 1988; Parker and Darby, 1995; Emerick *et al*., 1999). A number of factors, such as disinfection dose, water quality, and species of microorganisms targeted, determine the effectiveness of disinfection in water and wastewater. If the particulate matter concentration is high in water, an appreciable concentration of microorganisms are likely to be associated with particles and shielded from the disinfectant.

The goal of the research presented herein is to investigate and compare the disinfection effectiveness of UV irradiation and free chlorine for naturally occurring particle-associated coliform (PAC) and non-particle associated coliform (NPAC) in wastewater. If coliform bacteria that are associated with particulate matter can survive following practical doses of UV and chlorine, they are likely to survive the disinfection process encountered in a treatment plant. There have been a number of studies that investigated the effectiveness of UV or chlorine disinfection alone on PAC (Qualls *et al*., 1983; Berman *et al*., 1988; Parker and Darby, 1995; Emerick *et al*., 1999). However, direct comparison of UV and chlorine disinfection systems using the same wastewater source under similar laboratory conditions has not been previously reported. This is of particular importance since meaningful comparisons between disinfection systems, leading to effective treatment strategies, can only be made under identical laboratory protocols, and not between two unrelated studies.

The specific objectives of this study were to:

- investigate the inactivation of PAC and NPAC under UV and chlorine disinfection;
- compare the inactivation of UV and chlorine disinfection for PAC and NPAC;
- evaluate the effectiveness of physical and chemical extraction/separation methods for disrupting flocs and exposing PAC to disinfectants.

Materials and methods

Secondary effluent used in this study was collected from Durham Northside Wastewater Treatment Facility (Durham, USA). The samples were collected early in the morning, and transported to the laboratory within 20 minutes. Wastewater was kept at 4°C and all analyses were completed within 12 hours.

This study was divided into two phases. In Phase I, samples containing PAC and NPAC were inactivated using UV disinfection at doses ranging from 0–100 mJ/cm^2. Samples containing NPAC were obtained using three different methods: EGTA extraction, filtration, and blending followed by filtration. In Phase II, samples containing PAC and NPAC were inactivated using chlorine disinfection at CT values of 0–525 mg min/L. The dose-survival relationships and inactivation rates of PAC and NPAC, for chlorine and ultraviolet irradiation, were then compared.

Samples containing NPAC and PAC were prepared as follows. NPAC were obtained using three different methods: chemical extraction, filtration, and filtration combined with blending. For the first method (NPAC-1), EGTA was added to samples to give a final concentration of 1 mM from a working solution of 0.1 M EGTA, and the samples were blended for 1 minute at 3500 rpm. During blending, sample temperature in the blender increased by 1°C. A seven-speed laboratory blender (Model No. 34BL97, Waring Products, New Hartford; CT, USA) was used to blend the samples. In the second method (NPAC-2), wastewater was filtered through a 5 μm filter (Whatman Inc., Clifton, NJ, USA) to separate PAC from NPAC assuming the majority of PAC are associated with particles greater than 10 μm (Emerick *et al*., 1999) and will be held by the filter. In the third method (NPAC-3), wastewater was again filtered through the 5 μm filter but this time the filtrate was blended for 1 minute at 3,500 rpm to further minimize the possibility of PAC remaining in the sample. Unaltered wastewater (secondary effluent) was used for samples containing PAC since wastewater contains coliform that are naturally associated with particles. The coliform in wastewater exist in both PAC and NPAC states, but NPAC would be inactivated first during disinfection, and thus their presence would not preclude observation of the disinfection results for the PAC portion of the wastewater sample.

UV (Phase I) and chlorine (Phase II) disinfection were used to inactivate the NPAC and PAC in wastewater. In Phase I, the UV disinfection experiments, a sample size of 20 mL was placed in a petri dish and exposed to monochromatic UV light (low-pressure mercury vapor lamp) at a wavelength of 254 nm. The sample was stirred continuously during the irradiation. The incident irradiation was measured using a radiometer with a germicidal UV detector (Model No. IL 1700 SED 240, International Light, Newbury Port, MA, USA) calibrated at 254 nm to standards traceable to the National Institute of Standards and Technology (NIST). The average irradiance was calculated by correcting the incident irradiance for the spatial incident variation across the petri dish, the liquid absorbance of the sample, and the sample depth (Morowitz, 1950). Absorbance of the samples was measured using a Varian Cary 100 Bio UV-visible spectrophotometer (Varian Analytical Instruments, Walnut Creek, CA, USA). UV dose (mJ/cm^2) was calculated as the product of the average UV irradiance (mW/cm^2) multiplied by the exposure time (s), with UV doses ranging between 0 and 100 mJ/cm^2.

In Phase II, the chlorine disinfection experiments, wastewater samples having an initial

total chlorine concentration of 1 mg/L, 5 mg/L, 10 mg/L, and 15 mg/L were prepared using fresh household bleach (5.25% sodium hypochlorite, Clorox). The contact times studied were 5, 15, 30 and 45 minutes. Free chlorine and combined chlorine concentrations were determined using a combination of DPD ferrous titrimetric and DPD colorimetric methods (*Standard Methods for the Examination of Water and Wastewater*, 1995). 0.1% sodium thiosulfate (EM Science, Gibbstown, NJ, USA) solution was used to neutralize the chlorine in samples following disinfection.

Surviving coliform after the disinfection procedures were determined using the membrane filter (MF) method (*Standard Methods for the Examination of Water and Wastewater*, 1995) and m-Endo Agar LES medium (Difco Laboratories, Detroit, MI, USA) in triplicate for UV disinfection experiments and in duplicate for chlorine disinfection experiments. Average particle size and total particle number of the samples were determined in triplicate using a particle size counter (Model No. WG5-260, Met One, Grants Pass, OR, USA). The samples were diluted at a rate of 1:5 before the particle size measurement. Particle-free wastewater filtered through a 0.45 μm membrane filter was used to dilute the samples to avoid changes in the ionic strength and ionic composition of the samples. Thus, possible changes that may occur in the particle size distribution and particle number of the samples due to dilution were minimized.

Results and discussion

UV disinfection

In Phase I, samples containing NPAC and PAC were inactivated using UV disinfection at doses from 0–100 mJ/cm^2. As illustrated in Figure 1, PAC exhibited a slower inactivation rate and tailing, whereas NPAC were more easily and rapidly inactivated to beyond detection limits. Even after relatively high doses of UV irradiation, complete inactivation of PAC could not be achieved. These results indicate the likelihood that some of the coliform were associated with particles to an extent that they were completely shielded from UV light. The three samples that were treated to produce NPAC were easier to inactivate. No surviving coliform were found for NPAC at a UV dose of 90 mJ/cm^2 for the first sample (NPAC-1), which was obtained by EGTA extraction. The other two samples containing NPAC (NPAC-2 and NPAC-3) had no surviving coliform at a UV dose of 50 mJ/cm^2. Even though EGTA treatment increased the number of particles in smaller fractions and reduced the average particle size in NPAC-1 as presented in Table 1, some of the remaining particles were still large in size (>10 μm) and may be able to shield coliform.

Filtration, on the other hand, eliminated the majority of large particles in addition to other smaller particles, and reduced the possibility of PAC presence in samples NPAC-2 and NPAC-3 (see Table 1). This might explain why 90 mJ/cm^2 was required to completely inactivate NPAC-1, whereas a UV dose of 50 mJ/cm^2 was sufficient for complete inactivation in NPAC-2 and NPAC-3. Figure 1 also illustrates that log inactivation of NPAC-1, NPAC-2, and NPAC-3 were greater than the log inactivation of PAC at a given UV dose. This indicates that the disinfection effectiveness of UV on NPAC is appreciably greater than that of PAC. A 4-log reduction in the initial coliform density was accomplished at 30 mJ/cm^2 for all of the NPAC samples, however, 4-log reduction could not be accomplished for the sample containing PAC even after a UV dose of 100 mJ/cm^2.

The particle size distributions of the samples containing NPAC and PAC are presented in Table 1 in three different particle size ranges: 2–5 μm, 5–10 μm, and >10 μm. EGTA extraction (NPAC-1) increased and filtration (NPAC-2) decreased the total number of particles in all of the particle size ranges. When filtration was followed with blending (NPAC-3), an increase in the total number of particles in all of the particle size ranges was observed as compared to NPAC-2. Some of the particle size data were inconsistent with

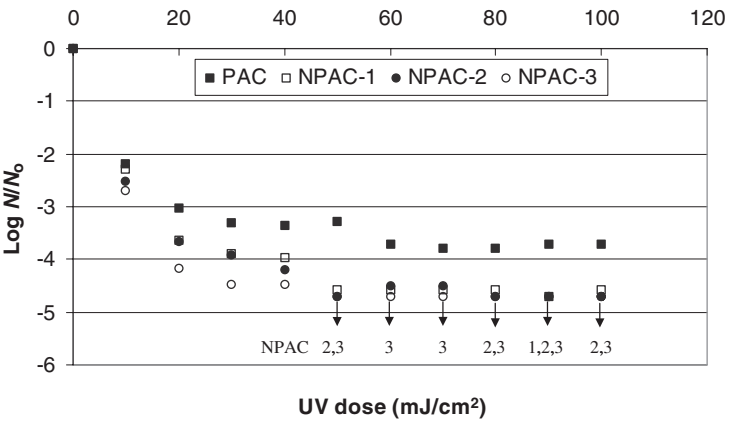

Figure 1 Each data point is the average of three replicates. Arrows indicate doses at which no coliform were detected

Table 1 Particle size distributions of the wastewater samples containing PAC and NPAC

Particle size range (μm)	Number of particles/mL			
	PAC	NPAC-1	NPAC-2	NPAC-3
2–5	8,645	11,495	4,625	5,385
5–10	3,265	4,430	790	935
>10	1,750	1,865	205	245

what would be expected from the floc disruption protocols. For example, blending increased the number of particles that were larger than 10 μm in NPAC-1 as compared to PAC and NPAC-3 as compared to NPAC-2, when it would be expected to have decreased. Possible explanations for this increase are as follows. First, the anomaly may be inherent to the problems associated with particle size counting (Chowdhury *et al*., 2000). Second, even though the samples may come from the same wastewater source they may exhibit variability in the number of particles and the particle size distribution. Therefore, it is possible to observe some discrepancy between the results. Nonetheless, the overall results from the particle counts and disinfection trials indicated that when large particles and flocs were broken into smaller pieces or filtered out using physical and chemical techniques, the inactivation rate of the coliform increased and little or no tailing in the dose response curve occurred.

Chlorine disinfection

In the second part of this study, samples containing NPAC and PAC were inactivated using chlorine disinfection. Based on the results from the UV study and the particle size analysis, the filtration method for isolation of NPAC (NPAC-2) was chosen to separate NPAC from PAC in the chlorine disinfection experiments. Chlorine was added to wastewater samples to give an initial total chlorine concentration of 1 mg/L, 5 mg/L, 10 mg/L, and 15 mg/L. The contact times used were 5, 15, 30 and 45 minutes. Free and combined chlorine concentrations remaining in the samples were measured at time periods throughout the experiment. The decay rate of chlorine was the same for both NPAC and PAC containing wastewater. Figure 2 illustrates the decay of the total chlorine during the first 45 minutes for various starting concentrations of total chlorine in PAC containing wastewater. Similar total chlorine decay rates were observed at all chlorine concentrations. Chlorine was completely consumed in the sample that contained 1 mg/L initial total chlorine. At higher starting chlorine concentrations (5, 10, and 15 mg/L), a rapid decay in the chlorine concentration

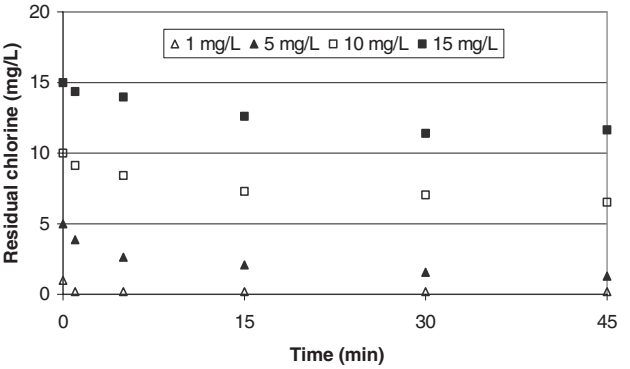

Figure 2 Residual total chlorine concentrations remaining in the samples at the end of each contact time during the disinfection experiment

took place within the first 5 minutes and slowed at longer contact times. The average total chlorine loss over the duration of the experiment was approximately 3.5 mg/L for the samples that had initial total chlorine concentrations of 5, 10, and 15 mg/L.

Figure 3 illustrates the inactivation of PAC and NPAC containing wastewater at chlorine doses ranging from 0–525 mg min/L. The initial total chlorine concentrations in the samples were 1, 5, 10 and 15 mg/L, and the contact times were 5, 15, 30 and 45 minutes. Chlorine doses were calculated by integrating the chlorine residual concentration over the contact time. The surviving number of coliform at a given initial total chlorine dose and contact time are given in Table 2 to supplement the data presented in Figure 3. Compared to the PAC containing wastewater, inactivation of the NPAC wastewater using chlorine disinfection was more rapid at doses below 150 mg min/L. The lowest chlorine dose required to disinfect NPAC completely was 58 mg min/L which was accomplished at 5 mg/L initial chlorine and 45 minutes contact time. However, some of the NPAC were still viable after exposure to chlorine doses of 109 mg min/L and 189 mg min/L when the contact times were only 15 minutes with a dose of chlorine at 10 and 15 mg/L, respectively (Figure 3). As in the case of UV disinfection, some of the PAC were also less susceptible to disinfection, and survived after exposure to a chlorine dose of 342 mg min/L at 15 mg/L initial chlorine and 30 minutes contact time. On the other hand, total inactivation of PAC was achieved at a lower chlorine dose of 293 mg min/L when the initial chlorine was 10 mg/L and the contact time was 45 minutes (see Table 2). Overall, total disinfection of PAC could only be

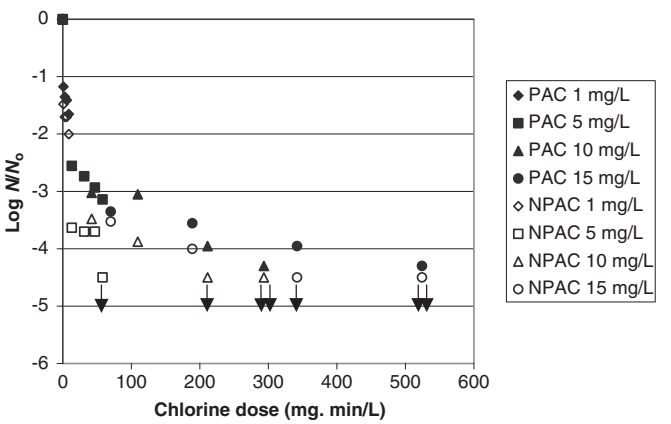

Figure 3 Dose-log survival relationship of PAC and NPAC under chlorine disinfection

achieved when the contact time was 45 minutes and the initial chlorine concentrations were relatively high (10 and 15 mg/L). Contact times shorter than 45 minutes resulted in the survival of PAC regardless of the initial chlorine concentration.

These results indicate that contact time plays an important role in determining the effectiveness of chlorine disinfection in wastewater, and chlorine dose alone may not be a good indicator of disinfection effectiveness for PAC. A lower chlorine dose with longer contact time is likely to be more effective on PAC than an identical CT achieved with a higher chlorine dose and shorter contact time. Microorganisms that are associated with particles or immersed in flocs are not likely to come into immediate contact with chlorine, therefore, while NPAC are inactivated quickly, PAC may remain unaffected. However, since wastewater flocs have a porous structure, chlorine will likely be able to reach the protected PAC given enough time. Since the inactivation of PAC does not take place immediately, it is important to assure that the initial chlorine concentration in wastewater is high enough to cover the chlorine demand of the wastewater and provide enough residual chlorine for the disinfection of PAC. In our experiments, when initial chlorine concentration was 1 mg/L and 5 mg/L in samples, total inactivation of PAC could not be achieved even after 45 minutes of contact time.

Comparison of UV and chlorine disinfection

The results of this study indicate that under prolonged contact times chlorine appears to be more effective than UV irradiation for the inactivation of PAC. Because the exposure times used in UV disinfection are much shorter than the contact times used in chlorine disinfection (and longer contact time gives more time and opportunity for chlorine to reach the protected microorganisms), these results are not unexpected. UV light cannot penetrate through particles (Loge *et al.*, 1999), and therefore microorganisms embedded in particles or flocs cannot be inactivated unless a direct light pathway exists between the target microorganism and the UV source.

From the results presented herein, it appears that some PAC can survive after being exposed to a UV dose of 100 mJ/cm^2, or a chlorine CT of 342 mg min/L. This is of particular importance for the following reasons.

1. If PAC can survive the high disinfectant doses used in this study, they may also survive typical disinfection doses encountered in a treatment plant [2–8 mg/L chlorine and 15–45 minutes contact time or 15–100 mJ/cm^2 UV dose (WEF, 1996)].

Table 2 Residual total coliform density remaining following various chlorine doses and contact times

Contact time (min)	Chlorine concentration			
	1 mg/L	5 mg/L	10 mg/L	15 mg/L
	PAC colonies/100 mL			
0	90,000 (±12,662)[1]	90,000 (±12,662)	90,000 (±12,662)	90,000 (±12,662)
5	6,000 (±354)	250 (±49)	85 (±7)	40 (±11)
15	4,000 (±1,061)	165 (±14)	80 (±18)	25 (±4)
30	3,500 (±354)	105 (±4)	10 (±0)	10 (±4)
45	2,000 (±0)	65 (±18)	ND[2]	ND
	NPAC colonies/100 mL			
0	151,000 (±8,185)	151,000 (±8,185)	151,000 (±8,185)	151,000 (±8,185)
5	5,000 (±1,414)	35 (±7)	50 (±6)	45 (±14)
15	3,000 (±1,060)	30 (±11)	20 (±4)	15 (±7)
30	3,000 (±0)	30 (±0)	ND	ND
45	1,500 (±354)	ND	ND	ND

[1] One standard deviation reported
ND, not detected

2. Research has shown that coliform bacteria are less resistant to physical and chemical disinfection compared to viruses (Havaelaar and Nieustad, 1985; Wolfe, 1990) and chemical disinfectants compared to cysts (Finch *et al*., 1995; Clancy *et al*., 2000). Therefore, the survival of PAC through a disinfection process may indicate the survival of other more resistant health related microorganisms in the treated water.
3. Since coliform bacteria are over 100-fold larger than viruses, it may be even more difficult to disinfect viruses associated with particles.

Conclusions

Based on the findings presented above, the following can be concluded.

- In wastewater, inactivation rate of non-particle-associated coliform is greater than the inactivation rate of particle-associated coliform under UV or chlorine disinfection.
- Under prolonged contact times, chlorine appears to be more effective for inactivation of particle associated coliform than UV irradiation.
- Contact time appears to be the most important factor in determining the effectiveness of chlorine disinfection of particle associated coliform. Thus, the chlorine dose alone may not be a good indicator of disinfection effectiveness in wastewater.
- Particle associated coliform can survive UV and chlorine disinfection at doses that are typically encountered in a wastewater treatment plant.
- EGTA extraction, as well as filtration and filtration followed by blending were successful in reducing particle-associated coliform in wastewater.
- Although UV is an effective technology for inactivation of non-particle associated microbes, complete inactivation in the presence of particles may not be practical.

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