Impact of flocculated particles on low pressure UV inactivation of *E. coli* in drinking water

Guo Liu, Robin M. Slawson and Peter M. Huck

ABSTRACT

This study investigated the impact of coagulated/flocculated particulate matter on low-pressure (LP) UV inactivation of spiked *Escherichia coli* under drinking water conditions using a standard bench scale collimated beam apparatus. The effect of floc particles in both coagulated river water and coagulated process (treatment plant) water was assessed. Laboratory grade water and uncoagulated river water were used as controls. The dose-response curves of spiked *E. coli* were determined at UV doses of 5, 10, 15, 25, and 40 mJ/cm^2. The results indicated that surface water particles (turbidity from 12 to 32 NTU essentially have no influence on UV inactivation of spiked *E. coli* if they are appropriately accounted for in the UV transmittance determination. However, the *presence of* floc particles formed by coagulation and flocculation led to significantly lower inactivation of *E. coli* by LP UV. A limited investigation of medium pressure UV inactivation undertaken as part of the study indicated that shielding effects for medium pressure UV warrant further investigation. The support for the inactivation results provided by the particle size analysis carried out suggests that criteria related to particle size may be very useful in addition to turbidity for UV regulation and operation. The results emphasize the need for UV disinfection/inactivation to be placed following filtration, because a sedimentation upset (i.e. where some floc particles may reach a downstream UV unit) could potentially compromise disinfection/inactivation.

Key words | bench-scale collimated beam, coagulation/flocculation, *E. coli* ATCC 11229, low-pressure, medium-pressure, UV inactivation

Guo Liu
Jacques Whitford Ltd,
3430 South Service Road,
Burlington, Ontario L7N 3T9,
Canada

Robin M. Slawson
Department of Biology,
Wilfrid Laurier University,
Waterloo, Ontario N2L 3C5,
Canada

Peter M. Huck (corresponding author)
NSERC Chairholder in Water Treatment,
University Research Chair,
NSERC Industrial Research Chair in Water Treatment,
Dept. of Civil and Environmental Engineering,
University of Waterloo,
200 University Ave., West Waterloo,
Ontario N2L 3G1,
Canada
Tel.: 519-888-4567, ext. 32707
Fax: 519-746-7499
E-mail: *pm2huck@uwaterloo.ca*

INTRODUCTION AND OBJECTIVES

Ultraviolet (UV) disinfection has been broadly utilized for secondary effluent from wastewater treatment facilities for meeting discharge regulations. Compared to chlorination, UV inactivation has been found to form few toxic residuals or disinfection byproducts (DBPs) that could be discharged to the receiving water body (Whitby & Scheible 2004). Since suspended solids are present in measurable amounts in secondary effluent from wastewater treatment plants, a considerable amount of research has been undertaken to investigate whether UV light can inactivate microorganisms (e.g. total coliforms as indicator bacteria) effectively in the presence of those suspended solids (Qualls *et al.* 1983, 1985; Parker & Darby 1995; Loge *et al.* 1996, 1999; Emerick *et al.* 1999, 2000). Results of this research in wastewater have indicated that clumping or particle association successfully shielded at least some of those microorganisms from UV irradiation.

In drinking water applications, UV disinfection/inactivation has been increasing recently since UV light was shown to effectively inactivate *Cryptosporidium* oocysts based on infectivity, even at very low doses (Clancy *et al.* 1998, 2000; Bukhari *et al.* 1999; Shin *et al.* 2001). An additional advantage of UV disinfection is minimal DBP formation. Liu *et al.* (2002) reported that low pressure (LP)

doi: 10.2166/aqua.2007.104

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and medium pressure (MP) UV light did not form significant DBPs at doses less than 500 mJ/cm^2. It should be noted that the regulated UV dose for drinking water disinfection is commonly 40 mJ/cm^2 (USEPA 2003).

In North America, a number of drinking water treatment facilities of various sizes supply potable water through watershed protection, water quality monitoring, and disinfection. These "unfiltered" systems meet the filtration avoidance criteria of the United States Surface Water Treatment Rule (SWTR, 40 CFR 141.71, USEPA 1979), which allows unfiltered turbidity of up to 5 NTU. The Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) (USEPA 2006) assigned the same UV dose requirement for both unfiltered and post-filtration systems. Additionally, the US Environmental Protection Agency (USEPA) only included inactivation data from studies where turbidity was less than 1 NTU (USEPA 2003).

An understanding of source water turbidity and particulate impact on UV disinfection/inactivation robustness is therefore crucial for UV application. Although this is true for unfiltered systems it is also true in terms of potential turbidity excursions in systems using filtration. Many researchers (Oppenheimer *et al.* 2002; Womba *et al.* 2002; Templeton *et al.* 2003, 2005; Batch *et al.* 2004; Passantino *et al.* 2004; Amoah *et al.* 2005) have investigated the impact of turbidity and particulates on UV disinfection performance in drinking water systems. In contrast to results obtained in wastewater, drinking water research has shown that the influence due to natural turbidity and particulates was insignificant for UV inactivation. However, significant shielding effects have emerged after the process of coagulation and flocculation in which it was hypothesized that some microorganisms would be partly or completely embedded in formed floc particles (Petri *et al.* 2000). Also, Templeton *et al.* (2005) have recently reported a study, including coagulation/flocculation, of LP UV inactivation of viral surrogates in synthetic water. Although the effect of coagulation and flocculation is relevant for treatment systems employing chemically assisted filtration, where the UV step is typically located after filtration, the present work relates more directly to situations where the UV step may be placed following a sedimentation tank or sludge blanket clarifier, perhaps because of site constraints in a retrofit situation.

Further research is necessary to provide greater knowledge and understanding of the impact of coagulated and flocculated particulate matter on UV disinfection under drinking water conditions. The goal of this research was to help fill this knowledge gap. The efficacy of low-pressure UV disinfection of *E. coli*, chosen to generally represent pathogenic bacteria, was investigated under the impact of coagulated and flocculated particulate matter in drinking water. The influence of uncoagulated particulate matter was also investigated to provide a point of reference. Limited investigations were also undertaken with medium pressure UV and the results are briefly discussed qualitatively. A secondary objective was to use particle size analysis (including particle imaging) to assist in interpreting the results from the UV inactivation experiments.

MATERIALS AND METHODS

Microorganisms

Since the concentration of pathogens of concern is usually low in drinking water sources, indicator bacteria were selected as target microorganisms for spiking into water samples and subsequent exposure to UV radiation. *E. coli* ATCC 11229 was chosen because it was well documented in terms of UV dose-response and potential photo/dark repair after irradiation (Zimmer & Slawson 2002).

A 16 to 18 hours culture of *E. coli* (ATCC 11229, Manassas, VA) in stationary phase (37°C) was selected for experimental purposes to closely represent cells in the environment. *E. coli* were suspended in 1× phosphate buffered saline (PBS, 0.01M, EMD™) and 1 mL aliquots of the suspension were used for spiking in water samples. For all tests the initial concentration was relatively stable at $\sim 10^6$ CFU/mL.

E. coli were enumerated by plate counts (MF technique) as described in Standard Method 9213D.3 (APHA *et al.* 1998), using mTEC agar (VWR) and GN-6 filters (0.45 μm, VWR). The plates were first incubated at 37°C for 2 hours and then at 44.5°C for 22 hours. Following this, the colonies characteristic of *E. coli* were counted.

Enumeration of viable cells was recorded as CFU (colony-forming units) per ml. Prior to filtering the samples, they were stirred (vortexed) to minimize clumping that

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might have occurred during the experiment, particularly during the coagulation trials. Although it is understood that a single colony may arise from small chains or clumps of cells rather than a single bacterial cell, the resuspension should have minimized this, and all colonies were observed to achieve similar sizes and morphologies during the incubation period. It was therefore accepted that comparisons could be readily made between the trials as excessive clumping would result in a "spreading" or oversized colony morphology, which would be noted as an estimated count. For ease of sample manipulation, smaller plates were used and consequently a statistically valid range of 10 to 100 colony-forming units per plate established.

In order to evaluate the possible need to extract bacteria from particulates, Liu (2005) conducted a preliminary investigation using local creek water containing particulates. None of the four methods tested (vortexing, sonication, or use of a blender or stomacher) showed a statistically significant difference in bacterial numbers compared to a control sample without any extraction method. For this reason and for the reason discussed in the previous paragraph, sample disruption was not undertaken prior to initiation of the bacterial enumeration procedure.

Sample preparation

As described below, five categories of water samples were prepared for UV irradiation, and UV dose-response of spiked *E. coli* was determined respectively. The sample of ultra-pure water was used as a control.

Ultra-pure water (MQ water)

A 1 ml aliquot of *E. coli* suspension was spiked into 500 ml ultra-pure water (Milli-Q UV Plus, 0.22 μm MilliPak-40 Ultra Pure Water system, MilliPore Corp., pH adjusted to 7.0). The 500 ml suspension was continuously shaken at 100 rpm (Model M49235, Thermolyne, USA) for 1 hour.

Filtered/unfiltered river water

River sediments or particles were obtained from raw surface water in the Grand River watershed. The sampling spot was the municipal intake location of the Mannheim Water Treatment Plant in the Regional Municipality of Waterloo.

A filtered river water sample was obtained by filtering 500 ml of river water (0.45 μm, GN-6, VWR). One ml aliquots of *E. coli* suspension were spiked into 500 ml of either filtered or unfiltered river water. Both of the suspensions were continuously shaken at 100 rpm (Model M49235, Thermolyne, USA) for 1 hour.

Coagulated river water

Alum stock solution was added as a coagulant in river water samples to form floc particles using a standard jar tester (Phipps & Bird Inc., VA). An optimum alum dosage (the dosage that gave the lowest supernatant turbidity following coagulation/flocculation/sedimentation) of 30 mg/L was determined through preliminary experiments and applied to form floc particles, as described below.

A 1 mL aliquot of *E. coli* suspension was spiked into a 500 mL river water sample, followed by adding 15 mL of alum stock solution (1000 mg/L). The solution was then mixed for 30 seconds at 100 rpm for coagulation, and then for 15 minutes at 30 rpm for flocculation. Thereafter the formed suspension with floc particles was transferred for UV irradiation.

Coagulated process water

Process water was obtained just upstream of the flocculation tank of the Mannheim Water Treatment Plant in the Regional Municipality of Waterloo, Ontario, Canada. The plant treats surface water from the Grand River with a design capacity of 72 million liters per day (MLD). At the time of sampling (January 2005), poly-aluminum chloride (PACl) was being used for coagulation (SternPAC; Eaglebrook, Inc. of Canada, Brantford, Ontario). Its formula is $Al_{13}(OH)_{20}(SO_4)_2Cl_{15}$ and is 10.3% ± 0.3% Al_2O_3 with a basicity of 51.0% ± 4.0%.

The sampling point was the upflow weir just prior to the flocculation tank, after the PACl had been dosed. The overflow of the weir was collected as coagulated process water and arrived in the laboratory 10 to 15 minutes after it was collected. Immediately upon arrival in the laboratory, a 1 mL aliquot of *E. coli* suspension was spiked into a 500 mL

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process water sample. The solution was then mixed for 15 to 45 minutes (based on the formation of visible floc) at 30 rpm for flocculation using a standard jar tester (Phipps & Bird Inc., VA). Thereafter the formed suspension with floc particles was transferred for UV irradiation.

UV irradiation

A standard bench scale collimated beam apparatus (Calgon Carbon Corp.) equipped with a replaceable LP (12 W) or MP (1 kW) mercury UV lamp was employed to irradiate the prepared water samples. The irradiation procedure followed the well-accepted laboratory protocol (Cabaj *et al.* 2001; Bolton & Linden 2003; USEPA 2003) for bench scale UV experiments.

The delivered UV doses were 5, 10, 15, 25, and 40 mJ/cm^2 for LP irradiation. UV doses of 5, 10, and 15 mJ/cm^2 were selected because a possible linear log inactivation (i.e. log reduction) was expected at lower doses; UV doses of 25 and 40 mJ/cm^2 were selected because the UV inactivation efficacy could be evaluated at doses commonly used in current regulations. The irradiation was duplicated for every dose. For each preparation (a total of 15, as each of the five categories of water samples was done in triplicate) a total of ten 8-mL aliquots were irradiated in a random manner. The lower doses also have some practical relevance because they could in fact occur in improperly maintained UV systems.

Limited medium pressure (MP) investigations were also conducted with what were believed at the time to be the same doses. However, subsequent to the completion of the investigation it was learned that a filter had been present in the radiometer. The filter did not interfere at 254 nm but did at other wavelengths. Therefore the MP doses actually applied were higher than intended. Since it was not possible to repeat the experiments, the MP results are discussed only qualitatively in this paper.

UV incident intensity was measured in mW/cm^2 at the center of the suspension's surface using a calibrated radiometer (Model IL 1700, International Light) with an SED 240 UV detector at 254 nm. UV exposure times were determined using a series of calculation spreadsheets (Bolton & Linden 2003; IUVA 2004) to deliver the designated UV doses. It should be noted that the absorbance of the particulates, as well as dissolved substances and the coagulant when present, were taken into account in the UV dose determination.

All samples were irradiated in the dark at room temperature and post-irradiated samples were stored in the dark before analysis (Sommer *et al.* 2000; Zimmer & Slawson 2002). After irradiation, *E. coli* were enumerated in duplicate series to determine the post-irradiated survival concentration.

Particle analysis

A bench scale laboratory Dynamic Particle Analyzer (DPA 4100, Brightwell Technologies Inc., 2004) was used to capture digital images of particles meeting user-selected criteria. The system software analyzes these images in real time to produce accurate particle size distributions.

A portion of the prepared samples (Section 3.2, 200 mL) was pre-filtered (to prevent plugging) using a 230-μm stainless steel screen (SS-8F-K4-230, Nupro Company, OH). The filtrates were then transferred to an accessory flask and analyzed as described in the instruction manual (Brightwell Technologies Inc., 2004).

Water quality parameters

The general water quality parameters measured included pH, dissolved organic carbon (DOC), turbidity, total suspended solids (TSS), and UV absorbance (UVA). pH was measured using a pH meter (Model 420A, Orion Research Inc.). DOC was measured by a total organic carbon (TOC) analyzer (Model 1010, OI Analytical, TX) in mg/L as described in Standard Method 5310B (APHA *et al.* 1998). Turbidity was measured using a portable turbidity meter (Model 2100P, Hach) in NTU. TSS was measured in mg/L as described in Standard Method 2540D (APHA *et al.* 1998). UVA is the product of UVA coefficient (cm^{-1}) and the path length (cm). The UVA coefficient in cm^{-1} of each sample was measured (254 nm for LP and 200–299 nm for MP UV trials) using a 1 cm quartz cell in a UV spectrophotometer (Hewlett-Packard 8453 UV-Visible Spectrophotometer, Canada) prior to irradiation. All the UVA measurements were conducted following the preparation of water samples (see Sample Preparation section) and immediately prior to UV irradiation.

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Statistical tests

Three data analysis tools in Microsoft Excel® were used as appropriate: 'Anova: Single Factor', 'Anova: Two-Factor with Replication', and 't-Test'.

RESULTS

General water quality parameters

General water quality parameters for the two sources used, river water and process water, are summarized in Table 1. Detailed data may be found in Liu (2005). The water (recall that the process water was also derived from the Grand River) was somewhat alkaline, of low to moderate turbidity and had a moderately high DOC.

Given the fact that turbidity ranged from 5.3 to 32 NTU in the water samples, UVA was probably overestimated by the direct spectroscopy technique due to non-differentiation between absorbance and scattering (For this reason, in previous work Christensen & Linden (2003) and Templeton *et al.* (2005) measured UVA using integrating sphere spectroscopy, which accounted for scattering effects by particles). For the present research, a spectrophotometer mounted with an integrating sphere sensor was not available. Therefore applied UV doses were likely higher than stated for those water samples containing particulates.

Dose-response of LP UV inactivation

For experiments involving low pressure UV, the response of spiked *E. coli* vs. UV dose is shown in terms of log inactivation for each of the five prepared samples (Figure 1). (The figure is split into two parts for clarity.) Each of the curves showed approximately 2 log inactivation (i.e. 99% removal) at 5 mJ/cm^2 without any statistical difference ($P > 0.05$). At higher UV doses, the presence of river water particles (in the absence of coagulation) seemed to have little or no impact on the inactivation of spiked *E. coli*. The three dose-response curves for MQ water (control), filtered river water (particle-free), and unfiltered river water (i.e. containing particles) were not statistically different ($P > 0.05$). These results are consistent with previous research (Linden *et al.* 2002; Oppenheimer *et al.* 2002; Batch *et al.* 2004; Passantino *et al.* 2004; Amoah *et al.* 2005) for seeding experiments involving spiked target microorganisms in drinking water sources under laboratory conditions.

In contrast, a significant shielding effect was observed when floc-associated *E. coli* were present as a result of coagulation and flocculation. The differences in log inactivation (statistically significant, $P < 0.05$) range from 0.7 to 1.8 log units in the dose range from 10 to 40 mJ/cm^2 when comparing coagulated river water to the other samples (i.e. MQ water, filtered/unfiltered river water). Similarly, statistically significant differences ($P < 0.05$) in log inactivation ranging from 0.6 to 1.4 log units in the dose range from 10 to 40 mJ/cm^2 were observed when comparing coagulated process water to the other water types. It is evident from Figure 1 that the lower range of the reported differences occurred at the higher doses. As was expected, the UV response of spiked *E. coli* showed no statistical difference ($P > 0.05$) at all doses when comparing coagulated river water and coagulated process water.

It was inferred that the floc-associated *E. coli* were less than 1% of total count because there was no statistical difference for all curves for less than 2 log inactivation, i.e. 99% removal of non floc-associated *E. coli*. However, the floc-associated *E. coli* could be a decisive factor in achieving desired higher log removals. This significant finding generally agrees with those of Malley (2000), Petri *et al.* (2000) and Templeton *et al.* (2003), all obtained for low-pressure UV. For instance, Templeton *et al.* (2003) found that MS2 bacteriophage enmeshed in coagulated clay particles (kaolin) partially escaped UV inactivation within a drinking water application. However more recently, in investigations of the inactivation of viral surrogates,

Table 1 | General water quality parameters of water samples

Parameter	River water	Process water
pH	7.6 ~ 8.4	7.8 ~ 8.1
Temperature, °C	4	8.7
Turbidity, NTU	12 ~ 32	5.3 ~ 17
TSS, mg/L	12 ~ 34	6.2 ~ 15
DOC, mg/L	6.1 ~ 6.9	Not measured
UVA, cm^{-1}	0.24 ~ 0.34	0.31 ~ 0.38

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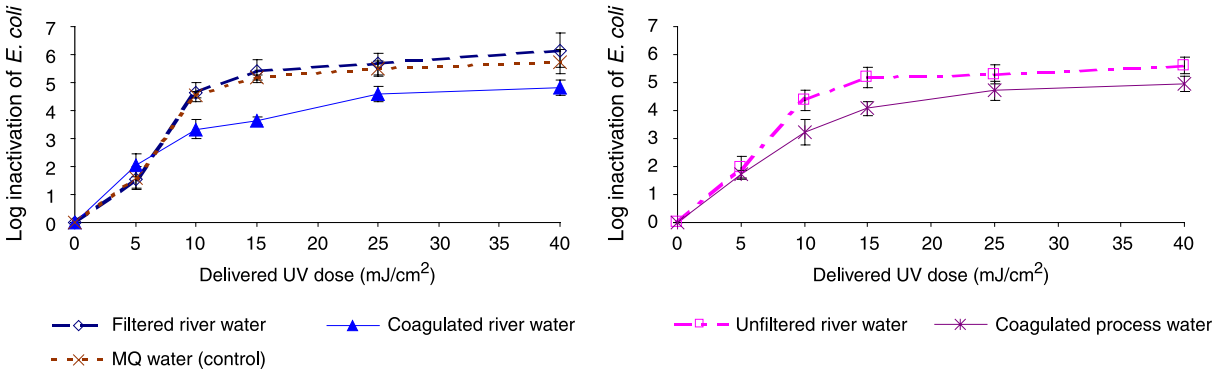

Figure 1 | Dose-response of spiked *E. coli* at LP doses of 5, 10, 15, 25, 40 mJ/cm^2. Error bars represent standard deviations of three to five replicate experiments.

Templeton *et al.* (2003) reported that the addition of coagulant did not produce a statistically significant decrease in log inactivation compared to trials without coagulant. Those authors also note that the particle-microorganism associations that occur in laboratory investigations (such as the present study) may not be representative of the attachment mechanisms in natural waters.

Dose-response of MP UV inactivation

Based on the results of the LP UV experiments, limited experiments were conducted to determine the dose-response of spiked *E. coli to medium pressure UV* in both MQ water and coagulated river water. The procedures of sample preparation and UV irradiation were the same as for the LP UV experiments.

As mentioned in the Materials and Methods section, it was discovered after the fact that the doses applied were higher than thought. This meant that these results could not be directly compared to the LP results. However, a valid comparison between the control sample and the coagulated river water for MP irradiation could still be made. The lack of a statistically significant difference between these two samples (except at one dosage, which we could not explain) (Liu 2005) does suggest that medium pressure UV may be less impacted by shielding. However, further investigation would be required to confirm this.

Particle size analysis

To assist in interpreting the experimental results presented above, particle size analysis was conducted. The particle size distribution (PSD) was determined for the following three sets of samples: (1) unfiltered river water before and after spiking *E. coli*, (2) coagulated river water before and after flocculation, (3) coagulated process water before and after flocculation. The photographic images of samples obtained by the apparatus were also examined.

The percentage PSD is shown in Figure 2 (each set of samples were analyzed in triplicate). The lower and upper boundaries for the particle counts were respectively 2 and 10 μm. The instrument has a lower particle size cutoff at 2 μm; the higher level was set at 10 μm based on the so-called critical particle size for the impact of a particulate matter (Qualls *et al.* 1985; Emerick *et al.* 1999; Dietrich *et al.* 2003). Each point shown represents an average for the interval (e.g. 3.5 μm represents the range from 3 to 4 μm).

The concept of critical particle size implies that any particle size below the critical value is not of concern while particles above the critical size are significant for the disinfection of particle-associated microorganisms. It is important to note, however, that in all the documented studies of critical particle size, the critical values were concluded or inferred from the divergence of dose-response curves before and after filtration by membranes of known pore size. Although the so-called critical particle size of 10 μm is established in the literature (based on wastewater studies) and provided a useful upper bound for these measurements, it is recognized that particles smaller than 10 μm may also have a shielding effect.

Since *E. coli* are in a size range of 1 μm (Black 1999), which is below the lower cutoff of the instrument, spiking these organisms had no observable effect on the PSD of unfiltered river water samples.

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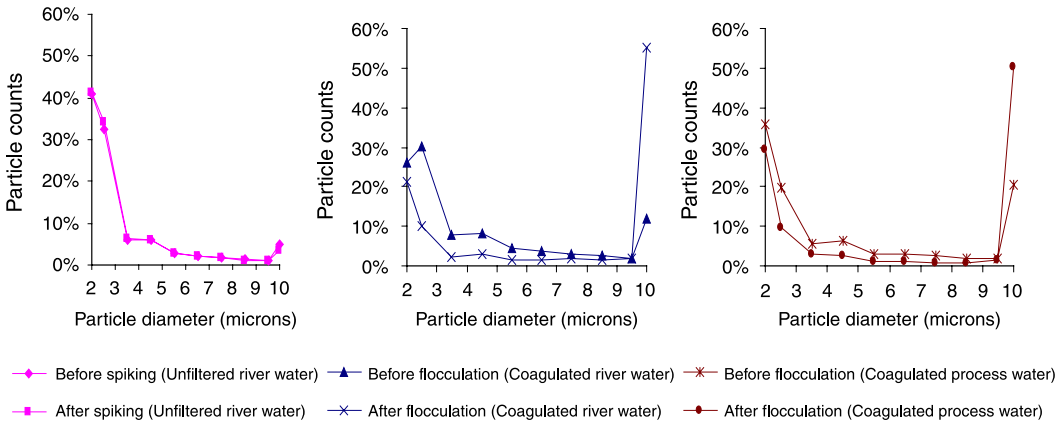

Figure 2 | Particle size distribution of samples prior to UV irradiation.

Conversely, the process of coagulation and flocculation tremendously changed the PSD of river water and process water, as would be expected. For river and process water samples, particles less than 3 μm decreased from 56% to 31% and from 56% to 39% of the total respectively, and particles greater than 10 μm increased from 12% to 55% and 21% to 50%, respectively. Thus, in the coagulated and flocculated samples, a substantial fraction of the particles was in the size range above which others have determined shielding effects can occur. This in turn suggests that particle size distribution may be a very useful parameter in addition to turbidity in assessing possible interferences with UV disinfection/inactivation in drinking water. Although the exact values obtained for the particle size distribution are dependent on the specific instrument used and on sample preparation, the present study indicates, in agreement with other researchers (e.g. Petri *et al.* 2000), the importance of integrating the impact of particle association into the proposed validation procedure for full-scale UV reactors (USEPA 2003).

A more than 3-fold increase was observed for the mean particle size in both flocculated samples (Table 2). The total number of particles decreased by one-third in the case of process water and by a factor of 14 in the case of river water, which further indicates the aggregation and enmeshment of particles with one another. Typical images of the suspensions before and after flocculation are shown in Figures 3 and 4 for coagulated river water and coagulated process water, respectively.

It should be noted that many visible floc and particulate aggregates were present in the process water sample even before flocculation (in Figure 4). This could be explained by the preliminary formation of floc particles during sample transportation after the coagulant was dosed at the plant. Much larger floc particles were present after flocculation in the laboratory.

Table 2 | Summary of changes in particle size distribution prior to UV irradiation

	Unfiltered river water		Coagulated river water		Coagulated process water	
Particle source	**Before spiking**	**After spiking**	**Before flocculation**	**After flocculation**	**Before flocculation**	**After flocculation**
Mean (μm)	4.7	4.3	6.3	21.8	10.5	33.7
Std dev (μm)	4.2	3.8	6.2	16.4	22.5	39.6
Total #/mL	100,000	88,700	231,000	16,100	18,900	11,700

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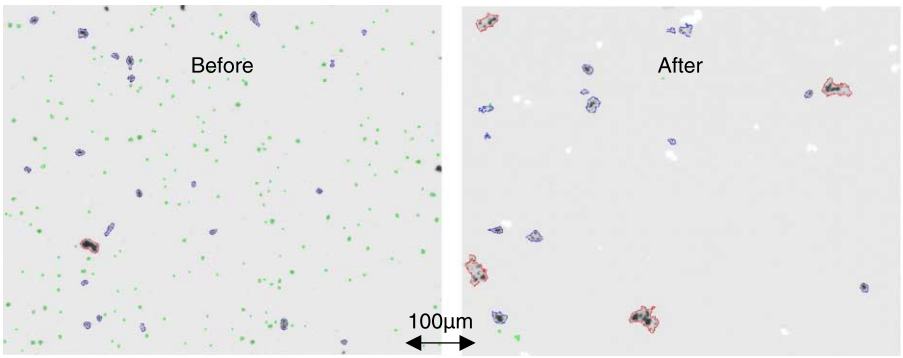

Figure 3 | Images of river water particles before and after flocculation.

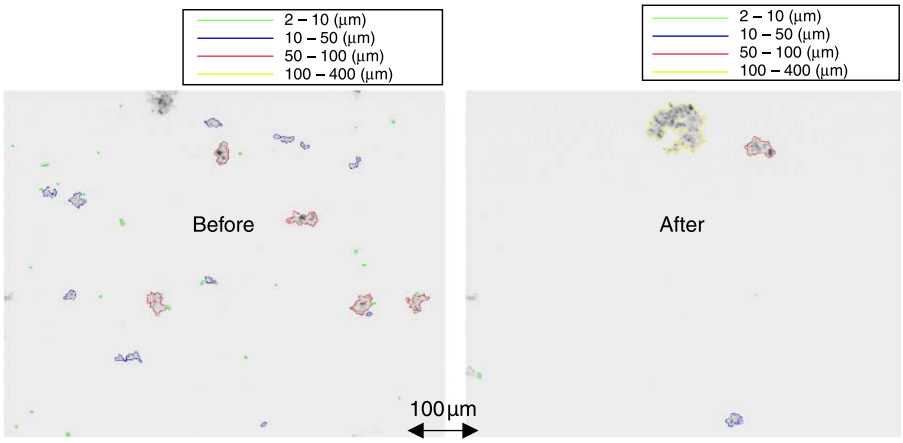

Figure 4 | Images of process water particles before and after flocculation.

CONCLUSIONS

The following conclusions can be drawn from this bench scale investigation (using a standard collimated beam apparatus) of the impact of particulate matter, with and without coagulation/flocculation, on low-pressure UV inactivation of a spiked organism, *E. coli*.

- In the absence of coagulation/flocculation, the presence of particles (turbidity from 12 to 32 NTU) in surface water had essentially no influence on UV inactivation of spiked *E. coli* if the particles are appropriately accounted for in the UV transmittance determination. These results are consistent with previous research.
- Less inactivation of *E. coli* was observed when floc particles were introduced through coagulation and flocculation. The average difference in log inactivation (statistically significant, $P < 0.05$) was 1.25 for coagulated river water and 1.10 for coagulated process (treatment plant) water when compared to MQ/filtered river/unfiltered river water at UV doses in the range of 10 to 40 mJ/cm^2. This potentially important difference in log inactivation was observed despite the inference from the data that the floc-associated *E. coli* were less than 1% of the total count.
- Particle size analysis verified the expected substantial shift in the particle size distribution after coagulation and flocculation, and supports the interpretations of the impact of coagulation/flocculation on UV disinfection. This result further suggests that particle size data may be of great value in addition to turbidity in setting criteria for UV disinfection processes.
- Although larger pathogens such as *Giardia* and *Cryptosporidium* should be less susceptible to shielding by flocs of a given size, this research indicates the potentially

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increased risk of pathogen passage through a treatment system under conditions of sedimentation tank upset, where floc particles might enter a downstream UV unit, if it were located prior to filtration.

- With medium pressure UV, initial experiments showed that the differences in inactivation between coagulated river water and the control sample (Milli-Q water) were not statistically significant except at one of the low doses (No explanation was available for this difference). These initial results suggest that, at least for bacteria, MP UV may be less sensitive to shielding effects. However this would need to be confirmed and warrants further investigation.

ACKNOWLEDGEMENTS

The authors would like to thank the staff at the Regional Municipality of Waterloo for providing access to sampling locations at the Mannheim Water Treatment Plant, and Brightwell Technologies Inc., for supplying the particle analysis apparatus and associated technical support. The assistance of Drs. Michele Van Dyke and William B. Anderson is appreciated.

Funding for this project was provided by the Natural Sciences and Engineering Research Council of Canada (NSERC) in the form of an Industrial Research Chair at the University of Waterloo. The current Chair partners include: American Water Services Canada, Brightwell Technologies Inc., the cities of Brantford, Guelph, Hamilton, Ottawa, and Toronto, Conestoga-Rovers & Associates Ltd., EPCOR Water Services, the Ontario Clean Water Agency (OCWA), PICA USA Inc., RAL Engineering Ltd., the Region of Durham, the Regional Municipalities of Niagara and Waterloo, Stantec Consulting Ltd., and Zenon Environmental Inc.

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First received 21 September 2006; accepted in revised form 21 December 2006

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