Inactivation of particle-associated viruses by UV

J. Y. Hu, X. N. Chu, P. H. Quek, Y. Y. Feng and X. L. Tan

ABSTRACT

Ultraviolet (UV) disinfection of bacteriophage MS2 in different waters containing particles was studied and a two-stage survival phenomenon was observed. It was found that turbidity had a negative impact on UV disinfection. In addition, the presence of fecal coliforms (*E. coli* C) was discovered to have an adverse effect on the virucidal efficiency of UV disinfection. It was also noted that the efficiency of UV disinfection was affected by particle size, number of particles, and particle size distribution. It was assumed that the indicator organisms in the water could be classified into two main categories, namely protected and less-protected to UV irradiation. In the less-protected group, viruses were assumed to exist in a dispersed state. In the protected group, on the other hand, viruses were assumed to exist in a particle-associated state. Based on this assumption, a model was derived for describing the measured inactivation of particle-associated viruses exposed to UV light. The results predicted by the proposed model were in good agreement with the experimental data.

Key words | bacteriophage, disinfection, modelling, particles, ultraviolet radiation, viruses

J. Y. Hu (corresponding author)
X. N. Chu
P. H. Quek
X. L. Tan
Division of Environmental Science and Engineering,
Center for Water Research,
National University of Singapore,
Singapore 119260
Tel.: +65-65164540.
Fax: +65-67744202.
E-mail: *esehujy@nus.edu.sg*

Y. Y. Feng
Faculty of Life Science and Technology,
Tongji University,
1239 Siping Road, Shanghai 200092,
China

INTRODUCTION

For decades, chlorination has been the most widely used technology for disinfection. However, safety concerns, formation of disinfection by-products and insufficient inactivation power towards resistant organisms are the most significant drawbacks of using chlorination. The said drawbacks have prompted efforts to develop alternative disinfection technologies. Ultraviolet (UV) irradiation has been found to be an attractive alternative disinfection technology. UV irradiation has several advantages over chlorination. These include: (i) it is an effective method against bacteria, viruses and protozoa; (ii) it could achieve required disinfection levels within a short period of time; (iii) it is an environmentally safe, non-chemical and physical process; and (iv) it does not produce toxic residuals or by-products (Kruithof & van der Leer 1990). In view of the various advantages associated with UV radiation, UV disinfection has been gaining popularity over the last two decades.

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A review of literature revealed that coliform bacteria have been used as the main indicator for evaluating UV disinfection efficiency in the past many years. It is also noted that maximum coliform concentration has been widely stipulated in effluent permit criteria by various water authorities. Recently, bacteriophages have been proposed to be better indicators for viruses than coliforms because: i) some of the bacteriophages resemble human viruses in size, morphology, structure and composition; ii) they are as resistant to water treatment and disinfection processes as coliforms; and iii) they are more resistant to UV disinfection than coliforms (IAWPRC 1991). Consequently, some countries or states have included bacteriophages as part of their microbiological standards in their effluent permit criteria (Levine *et al.* 1997).

It has been known that many of the waterborne microorganisms of interest (e.g., coliform bacteria and

viruses) are present in either dispersed (i.e., not bound to other objects) or particle-associated states (i.e., bound to other objects such as mineral or organic matters, bacteria or cellular debris). Dispersed microorganisms are more easily inactivated as they are readily exposed to UV light. In contrast, it is relatively less effective to disinfect particle-associated organisms as they may not be readily exposed to UV irradiation. In view of this, various empirical UV disinfection models have been developed to describe the response of coliform bacteria associated with particles (Severin *et al.* 1984a, b; Qualls & Johnson 1985; USEPA 1986; WPCF 1986; Scheible 1987; Loge *et al.* 1996). However, these models may not be applicable for modeling viruses due to size differences between bacteria and viruses (bacteria are approximately 2 orders of magnitude larger than viruses) (Emerick *et al.* 2000). As a result, there is no model currently available that could realistically describe the inactivation of particle-associated viruses. In view of this, the objective of this study was therefore to develop a UV disinfection model useful for describing the inactivation of particle-associated viruses in water. A commonly used viral indicator, bacteriophage MS2, was adopted in this study for investigating the behavior of disinfecting particle-associated viruses via UV irradiation.

MATERIALS AND METHODS

MS2 (ATCC 15597-B1) was spiked into 30 litres of tap water, reservoir water, and secondary effluent, respectively. The tap water was obtained from the laboratory. The reservoir water was collected from a local reservoir while the secondary effluent was obtained from a domestic wastewater treatment plant. The turbidities of all water samples were measured before use using a HACH 2100P turbidimeter (Hach Co, Loveland, Colo.). A particle size analyzer (LS 230, Coulter. Miami, Flo.) was used to measure the size distributions of particles present in waters. The characteristics of the various waters used in this study are summarized in Table 1. Other types of water samples were also prepared by adding different masses of silica colloids (Snowtex 20L, with particle size ranging from 0.04 to 0.05 μm, Nissan Chemical Co, Tokyo, Japan) or different number of *Escherichia coli* C (ATCC 13706) into the tap water. *E. coli* C was propagated in tryptic soy broth (TSB) medium and enumerated on TSB agar plates. The MS2 spiked water was then subjected to UV irradiation at different contact times using a contact UV system (TrojanUVMax™ Model B, Trojan Technologies Inc., Canada). This UV apparatus comprised a stainless steel outer housing and an inner quartz tube. The water was passed between the outer steel housing and inner quartz tube during UV disinfection. A low pressure mercury lamp was placed in the interior of the quartz-stainless steel cylinder to provide UV light exposure to the passing fluid. The average intensity of the UV lamp was 40 mW cm^{-2}. A schematic flow diagram of the system is shown in Figure 1. Concentrations of MS2 bacteriophage were quantified using the double agar layer method (Adams 1959) before and after UV disinfection. *E. coli* (ATCC 15597) was used as the host bacteria on TSB agar plates.

RESULTS AND DISCUSSION

The results obtained from disinfection studies on particle-associated MS2 in water samples with different turbidity,

Table 1 | Characteristics of waters tested

	Tap water	Reservoir water	Secondary effluent
pH	7.74	7.84	6.61
Turbidity (NTU)	0.3	4.2	2.1
Total Coliforms (CFU/ml)	N.D.	N.D.	13500
Fecal Coliforms (CFU/ml)	N.D.	N.D.	6500
HPC (CFU/ml)	390	700	520000
COD (mg O_2/ml)	1.9	17.3	29.3
TKN (mg/ml)	N.D.	0.7	4.0
Particle size range (μm)	0.0464–0.0734	38.3–222.4	22.8–179.2
Avg. particle diameter (μm)	0.0591	131.8	95.1

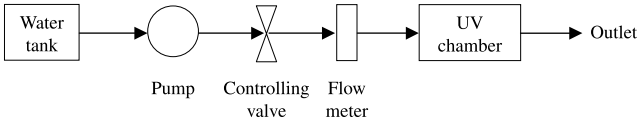

Figure 1 | Schematic flow diagram of UV disinfection system.

bacteria number, and different water matrices are given in Figures 2 to 4 as a function of contact time.

As UV intensity was held constant in the experiment, contact time could be used as an indicator for the UV dose. It is noted from Figures 2 to 4 that disinfection kinetics could be clearly demarcated into two phases. This observation suggested that, some viruses were more seriously affected by UV irradiation (this fraction of viruses represented the dispersed group) whereas others were less affected by UV irradiation (this fraction of viruses represented the particle-associated group). In view of this, the survival ratio of viruses could be expressed by using two exponential expressions as suggested by Shayeb *et al.* (1999) and Kowalski *et al.* (1998). The dispersed group of viruses, not being covered by particles or other matters, were directly exposed to UV light. As a result, the killing mechanism for this group of viruses followed the Chick's Law (Chick 1908), which exhibited an exponential kill according to a first order reaction or single-hit kinetics.

$$N = N_o \exp(-k_1 t) \quad (1)$$

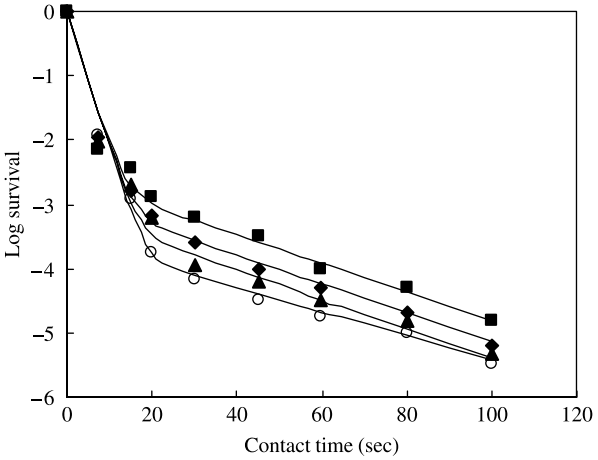

Figure 2 | Contact time-survival curves for MS2 in water samples with different turbidity. The turbidity was caused by adding silica colloids. Experimental results were indicated by turbidity of 2.08 (○), 4.29 (▲), 5.53 (◆) and 8.00 NTU (■). Model results (—) were calculated from Equation (3).

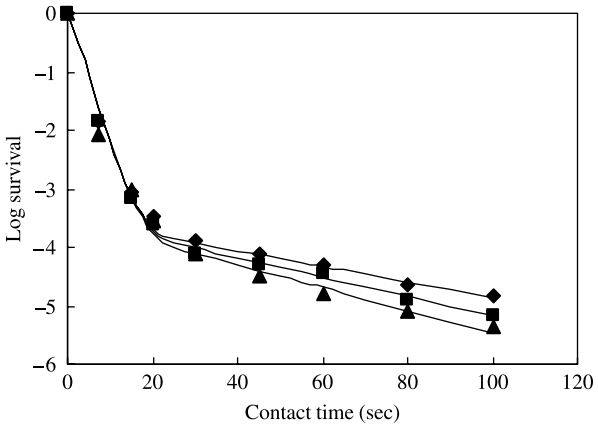

Figure 3 | Contact time-survival curves for MS2 in water samples with different *E. coli* C number. Experimental results were indicated by *E. coli* C number of 2×10^8 (▲), 1×10^9 (■), and 5×10^9 cfu (◆). Model results (—) were calculated from Equation (3).

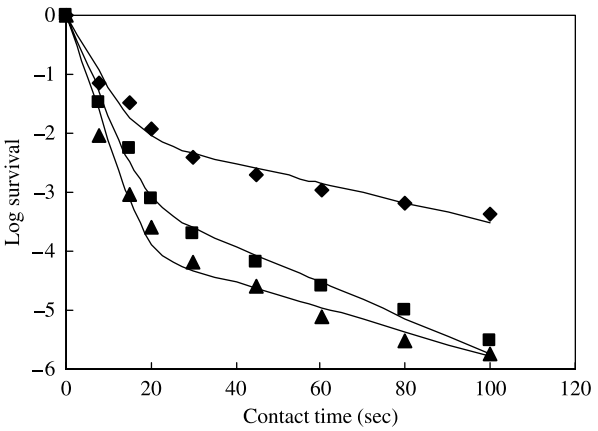

Figure 4 | Contact time-survival curves for MS2 in different water matrices. Experimental results were indicated by tap water (▲), reservoir water (■), and secondary effluent (◆). Model results (—) were calculated from Equation (3).

The particle-associated group of viruses, being shielded or partially shielded by particles (such as mineral or organic matter, bacteria or cellular debris in water samples), would undergo the same kind of inactivation but with a different inactivation rate, k_2. Accordingly, the number of coliphage could be expressed as:

$$N = N_o \exp(-k_2 t) \quad (2)$$

This fraction of viruses could even be virus aggregation resulting from UV irradiation (Blatchley *et al.* 2001). By considering a σ fraction of the population characterized by k_1 inactivation rate, and $(1 - \sigma)$ by k_2, the following

expression could be obtained:

$$\frac{N}{No} = \sigma\exp(-k_1 t) + (1-\sigma)\exp(-k_2 t) \quad (3)$$

The inactivation rate of dispersed viruses, k_1, is an intrinsic characteristic of a given virus. It has been believed that k_1 would not change with water matrices containing the same kinds of particles. However, the behaviour of k_2 would be less obvious because the effect of blockage or covering by other substances in water is unknown. Thus, both k_2 and σ would be likely to be subject to changes. The intensity of UV light was not included in the model because a constant intensity had been used in this study.

By applying the Least-Squares method, one is able to obtain a best-fitted log survival *vs* contact time curve. Error or discrepancy can be expressed as follows:

$$S = \Sigma[(\text{computed value} - \text{experimental value})^2] \quad (4)$$

In the least-squares method, it is required that S be minimized. This requirement could be achieved by determining the appropriate values of k_1, k_2 and σ that would minimize S via an optimization algorithm.

The experimental and predicted (based on Equation (3)) values of MS2 inactivation results associated with silica added water samples with different turbidity are shown in Figure 2. The values of the model parameters used in Equation (3) are presented in Table 2. It is noted from Table 2 that the value of k_1 was indeed constant which confirmed the prior assumption that k_1 would not change with water matrices containing the same kinds of particles. The value of σ (the fraction of dispersed virus) was noted to decrease with increasing levels of water turbidity. As water turbidity was adjusted by adding silica colloids of constant size (0.04–0.05 μm), a higher turbidity would indicate a larger number of particles which in turn would result in higher amounts of particle-associated viruses and hence a lower σ value.

The experimental and predicted (based on Equation (3)) values of MS2 in water with different numbers of *E. coli* C are shown in Figure 3. The values of model parameters used in Equation (3) are presented in Table 3. In this case, k_1 was still a constant, and σ changed insignificantly. In contrast, the value of k_2 reduced significantly when the number of *E. coli* C in water was increased. It is also noted that the values of k_2 in Table 3 were much lower than the corresponding values of k_2 shown in Table 2. As the size of *E. coli* C (2 μm) is larger than silica colloids (0.04–0.05 μm), *E. coli* C could protect a virus and render it more difficult to inactivate the protected virus. Consequently, k_2 in *E. coli* C added water was lower than k_2 in silica added water. As a result, k_2 decreased when a larger number of *E. coli* C was added into the water.

The experimental and predicted (based on Equation (3)) values of MS2 in different water matrices are shown in Figure 4 and the corresponding values of model parameters are given in Table 4. It is noted from Table 4 that k_1, k_2 and σ were quite different in different water matrices. The corresponding ranges of turbidity and particle size in three water matrices were also quite different. Tap water contains the least amount of suspended particles, with no detectable coliforms and much smaller particle sizes than the other water types. The reservoir water has higher turbidity and larger particle sizes than secondary effluent, which most likely is due to the fact that it is exposed to the atmosphere and contains some grit particles. Secondary effluent contains coliforms in the range of about 10^5 CFU/ml, but has slightly smaller particle sizes than the reservoir water. As the suspended particles present in different water

Table 2 | Model parameters for MS2 inactivation in silica added tap water with different turbidity

Turbidity (NTU)	2.08	4.29	5.53	8.00
K_1 (s^{-1})	0.488	0.488	0.488	0.488
K_2 (s^{-1})	0.044	0.053	0.052	0.052
σ	0.9997	0.9992	0.9987	0.9973

Table 3 | Model parameters for MS2 inactivation in *E. coli* C added tap water

E. coli C (cfu)	2×10^8	1×10^9	5×10^9
Turbidity (NTU)	0.3	0.35	0.73
K_1 (s^{-1})	0.5	0.5	0.5
K_2 (s^{-1})	0.045	0.038	0.031
σ	0.9997	0.9997	0.997

Table 4 | Model parameters for MS2 inactivation in different water matrices

	Tap water	Reservoir water	Secondary effluent
Turbidity (NTU)	0.3	4.23	6.61
Particle size (μm)	0.046–0.073	38.3–222.4	22.8–179.2
K_1 (s^{-1})	0.488	0.4	0.3
K_2 (s^{-1})	0.047	0.07	0.038
σ	0.9998	0.998	0.986

matrices consisted of a mixture of particles of various sizes, the different k_1 values obtained from the three water matrices could be attributed to the different particle size distributions. The particle size in tap water was approximately 3–4 orders of magnitude smaller than the size of particles in reservoir water and secondary effluent. Therefore, most of the UV light was irradiated on dispersed virus, which resulted in highest k_1 in tap water. The overlap of particle size ranges in reservoir water and secondary effluent made the comparison of their k_1 values difficult without knowing the distribution pattern. This indicated that the effectiveness of UV disinfection was affected not only by particle number or particle size, but by particle size distribution also.

CONCLUSIONS

Ultraviolet disinfection of bacteriophage MS2 in different waters containing particles indicates that it exhibits the characteristics of a two-stage survival phenomenon, wherein the first stage represents a rapid inactivation which corresponds to dispersed virus inactivation, and the second represents a slow inactivation which corresponds to particle-associated virus inactivation. Disinfection of viruses in particle-containing waters could be modelled as a reaction on a two-group population with different resistances to UV. The results predicted by the model are in good agreement with the experimental data. The presence of turbidity and coliform bacteria adversely affects the virucidal efficiency of UV disinfection. It is also noted that the efficiency of UV disinfection is affected by particle size, number of particles and particle size distribution.

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