

The Stability of Bacteriophage MS2 used in a UV Reactor Validation Study

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ABSTRACT

Bioassay validation of UV reactors in the United States is performed using bacteriophage MS2 or *Bacillus subtilis* spores. The USEPA draft UV Disinfection Guidance Manual indicates that the challenge microorganism concentration should be measured within 24 h of collection. Validation typically involves several consecutive days of field work and can yield up to 200 samples daily. Given that each sample requires multiple dilutions and triplicate plating, the number of plates analyzed per day can easily surpass 1,200. For most laboratories, plating this many samples within 24 h exceeds the lab's capacity and presents numerous logistical challenges. To address the issues of sample holding time and sample integrity, a subset of samples from a reactor validation study were analyzed within 30 h of collection and for several days thereafter. Results demonstrated that MS2 remained stable over time and suggest that the recommended 24 h holding time should be reconsidered.

INTRODUCTION

The USEPA's Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) (USEPA 2003a) requires utilities that intend to use UV disinfection for controlling pathogens to perform validation testing of their UV reactors. Testing is conducted by the UV manufacturer or by the utility at either a UV test facility or on-site at the water treatment plant (WTP) where the reactor would be installed and operated. UV reactors are currently validated using either bacteriophage MS2 or *Bacillus subtilis* spores as challenge microbes. Bioassay validation protocols are described in the USEPA draft UV Disinfection Guidance Manual (UVDGM) (USEPA 2003b), the German DVGW Standard W294 (Hoyer 2000) and the NWRI/AwwaRF UV guidance manual (NWRI 2003).

Bioassay validation of reactors with capacities of up to 40 million gallons per day (MGD) can require viral seed concentrations approaching 10^{16} organisms per day, with enumerative assays using up to 5,000 Petri dishes per validation. Large numbers of samples (i.e., 200) may be collected on any given day of testing and each sample is assayed with several replicates. The UVDGM indicates that the challenge microorganism concentration should be measured within 24 h of collection. Given the scenario of 200 samples per day, and multiple dilutions of each sample in triplicate, the number of plates required to analyze all the samples can be as high as 1,500.

For most laboratories, plating this many samples within 24 h exceeds the lab's capacity and presents numerous logistical challenges.

The capacity issue is compounded given that field testing occurs for several consecutive days and that some unknown number of samples may need to be re-assayed to obtain counts within the statistically valid range (plates having plaque forming units (pfu) ranging from 20-200). In addition, the dose response of MS2 must be determined at the bench scale using a collimated beam (CB) apparatus to establish the microbial inactivation kinetics for a given water (Bolton and Linden 2003). In effect, the CB apparatus is used to calibrate the challenge organism's response to UV. CB tests are performed in duplicate each day of reactor testing. CBs generate additional samples that must be assayed within 24 h. Hence, given the enormity of large scale reactor validation, sample holding time and sample integrity become critical issues.

To address the issues of sample holding time and integrity, we conducted a study wherein a subset of reactor samples were assayed within 30 h of sample collection and for several days thereafter to determine the stability of MS2 over time. Inlet and outlet samples as well as bench scale CB samples were selected to allow comparison of UV-treated versus UV-untreated conditions. Another aspect of MS2 stability examined was whether or not MS2's response to UV changes as a function of time. To address this question, the CB dose response curves of MS2 exposed to UV on Day 1 were compared to dose response curves of MS2 exposed to UV on Day 7. Lastly, two stocks of high titer phage [10^{11} plaque forming units (pfu) per mL] were assayed several times over the course of an eight month period to determine if the concentration of MS2 in each stock was stable.

MATERIALS AND METHODS

Preparation of MS2 Stocks

Bacteriophage MS2 (ATCC#15597-B1) was propagated using a large volume liquid culture method in *E. coli* HS(pFamp)R (ATCC#700891). Phage were propagated in 6 L batches and the titer determined within 24 h of production. Batches with an acceptable titer ($\geq 2 \times 10^{11}$ pfu/mL) were pooled into a single 20 L volume, mixed, redistributed in 1 L polypropylene bottles, retitered, and stored in a dedicated refrigerator at 4°C. Phage stocks were shipped by overnight express to the test facility and were stored at 4°C. All

phage used in the validation study was less than 2 months old. Viral stocks were diluted with carrier water prior to injection in all but the highest flow and target dose tests.

Enumerative MS2 Assay

Samples were analyzed by the double agar-layer method (Adams 1959). In brief, serial 10-fold dilutions of the samples in phosphate buffered water (PBW) were prepared. The appropriate volume of sample, based on predicted inactivation and spike dose level, was added to a tube of tempered overlay agar containing 100 μ L of *E. coli* Famp host. The tube was poured onto a plate of bottom agar (Tryptic Soy Agar supplemented with streptomycin and ampicillin) and allowed to harden for 10 min. Samples were plated in triplicate, inverted, and incubated at 35°C for a minimum of 14 h. Negative controls (host bacteria with PBW) were plated at the beginning and end of each run, and at intervals in between depending on the length of the plating run. Plaque counts from dilutions in the countable range were recorded and the concentration of phage were calculated as pfu/mL.

Sample Generation, Selection, Collection and Storage

The water source used in this study was chlorine free groundwater with a UV transmittance (UVT) greater than 98% (1 cm). Lignin sulfonate was added as a UV absorber to decrease the UVT to >75%. MS2 phage were injected into the inlet of a UV reactor upstream of static mixers. A sample port between the mixer and UV reactor served as the inlet sample site. The outlet port was situated downstream of the reactor and a second static mixer.

Two sets of UV reactor inlet/outlet samples (101/401 and 105/405) were collected in triplicate in sterile 15 mL polypropylene tubes and were stored on ice in a cooler immediately after collection. Additional seeded inlet (untreated) samples were collected in sterile 250 mL polypropylene bottles and stored in the same manner. These larger sample were used for CB experiments which were conducted at the laboratory. Samples were shipped by overnight express courier to the laboratory. Upon receipt, samples were logged in and stored at 4°C. All samples were analyzed within 30 h of collection and for several days thereafter.

UV Irradiation: UV Dose Response Determinations

A CB apparatus which delivers a controlled UV dose to a stirred suspension of microorganisms was used to determine the UV dose response of MS2. UV dose delivery to the suspension was controlled by varying the exposure time using a solenoid-controlled shutter. A non-reflective PVC tube (6.4 cm ID x 50 cm length) was used to ensure the UV light incident onto the suspension was parallel. Test solutions were mixed constantly, with care taken to minimize air bubbles and vortices. Prior to irradiating any organisms, the fluence rate was determined using the method described by Bolton and Linden (2003). Incident UV irradiance was measured using two radiometers (International Light model IL1400A/SEL 240) and Gigahertz Optik X911. Both instruments were calibrated at 254 nm, traceable to NIST standards, within 12 months of testing. The UV dose response of MS2 phage was

measured using the 250 mL sample taken at the reactor inlet as described above. Exposures of sufficient duration were made to provide the following UV doses: 0, 10, 20, 40, 60, 80, 100 mJ/cm².

Sample Assay Schedule

The two sets of UV reactor inlet and outlet samples (101/401 and 105/405) were assayed everyday for 6 days (101/401 were also assayed on Day 8), then held for 2 weeks and re-assayed on Day 20. The CB samples associated with reactor samples 101/401(CB1) were assayed on Days 1, 3, 5, 7 and 16. CB1 samples irradiated with 80 and 100 mJ/cm² were not plated on Day 16 due to insufficient sample volume. Two other distinct sets of CBs (CB75 and CB92) were exposed and plated on Day 1. A second set of sub-samples from these were also exposed and assayed on Day 7. The high titer MS2 stocks were sub-sampled and assayed intermittently over the course of an 8 month period.

RESULTS

The mean viral concentrations for the two sets of UV reactor inlet and outlet samples are shown in Figure 1. Data for the CB samples are presented in Figures 2 and 3. Figure 2 shows the mean viral concentrations of the exposed and unexposed CB samples over time while Figure 3 shows the resulting log viral inactivation [$\log(N/N_0)$] at each of the UV doses applied at Days 1, 3, 5, 7 and 16.

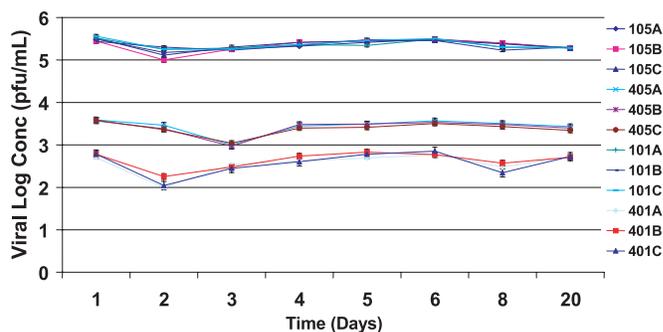


Figure 1: Viral Log Concentration of UV Reactor Inlet (101/105) and Outlet (401/405) Triplicate Samples Over Time.

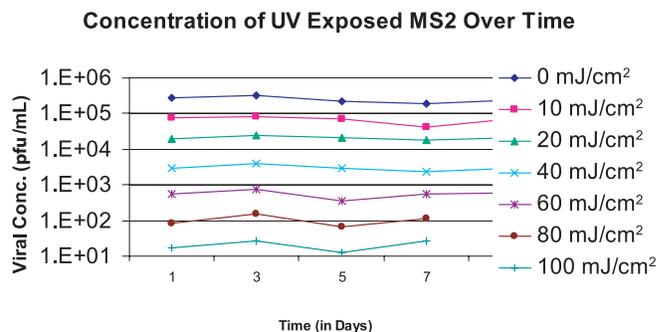


Figure 2: Viral Concentration of CB1 Samples Over Time

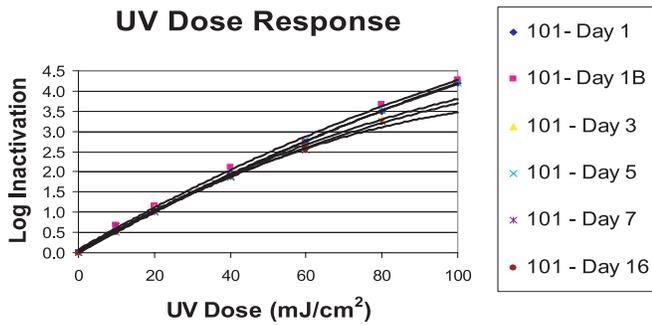


Figure 3. Log Inactivation of CB1 Exposed Samples as Analyzed Over Time.

Results from CB samples (CB 75 and CB92) that were independently exposed and assayed on Day 1 and Day 7 are presented in Figures 4 and 5.

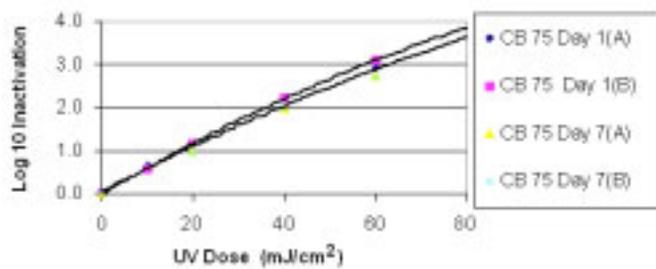


Figure 4. CB Sample 75 Exposed on Day 1 and Day 7, analyzed immediately after exposure.

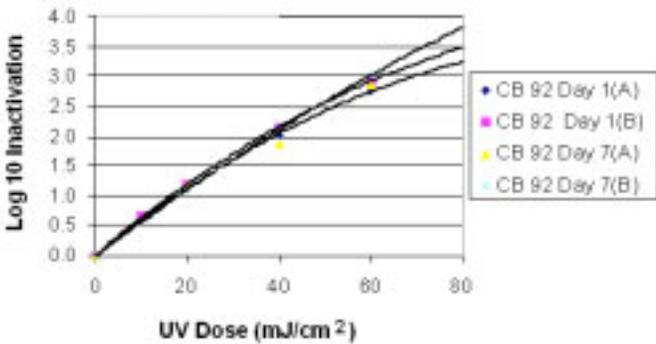


Figure 5. CB Sample 92 Exposed on Day 1 and Day 7, analyzed immediately after exposure.

Two high titer MS2 stocks were assayed numerous times over the course of 8 months. The viral log concentrations are presented in Figure 6.

DISCUSSION

The MS2 bacteriophage concentrations in both inlet and outlet samples appeared to remain stable over the course of this study, or approximately 3 weeks. Outlet samples showed more variability than inlet samples, however the concentrations after 20 days were similar from the concentrations on Day 1, as shown in Figure 1.

Viral concentrations in CB1 samples also appeared to remain fairly constant as illustrated in Figure 2. Moreover, the data suggest that viral concentrations in samples irradiated with UV behave similarly to samples that have not been exposed to UV. That is, MS2 infectivity over time is constant following exposure to UV. Figure 3 presents log inactivation data of a CB sample exposed to various UV doses analyzed out to 16 days. These data again illustrate the stability of MS2 in these UV-exposed samples.

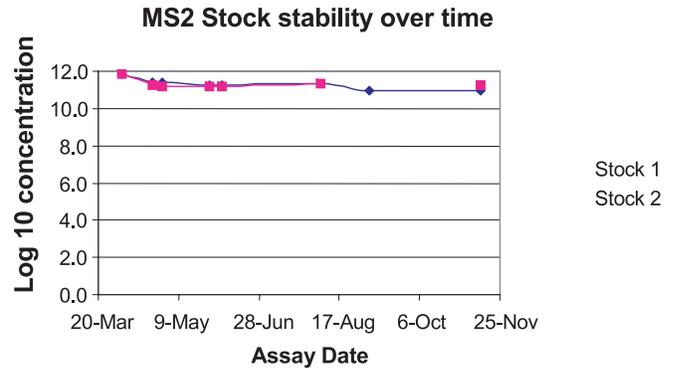


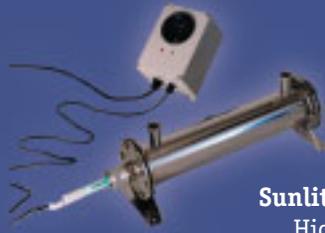
Figure 6. Two MS2 High Titer Stock Concentrations Over Time.

Results from CB samples exposed initially and one week later (CB75 and CB92) suggest that the dose response curves of MS2 do not change as a function of time. As Figures 4 and 5 demonstrate, the dose response curves of CB experiments performed on Day 1 are very similar as those performed on Day 7.

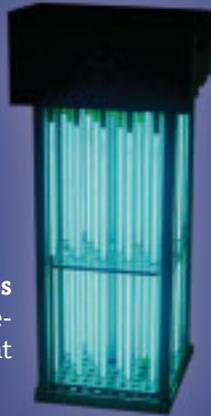
Two high titer MS2 stocks that were monitored intermittently over the course of an eight month period remained relatively stable. In general, titers exhibited an initial decrease by approximately one half log, then stabilized for the remaining 8 month period. Our laboratory routinely propagates phage in large batches and typically uses these batches in the field within 3 months of propagation. These data suggest that an even longer shelf life is acceptable for MS2.

The results from this study indicate that MS2 remains stable over time in the samples analyzed for UV reactor validation. Caution is recommended in approaching any validation, as others have noted that certain water qualities may negatively affect this stability (Petri et al. 2000). Analyses of samples collected from a different reactor validation are underway which indicate phage concentrations may not always be as stable as the above findings demonstrate.

The implications regarding sample holding time and sample integrity are significant considering the laboratory's logistical challenges of analyzing such a large number of samples within a short timeframe. Furthermore, for those samples that are re-assayed to obtain plaque counts within a statistically valid range - and are therefore not analyzed within the recommended 24 h holding time - the quality of data does not appear to be compromised in the study presented above. However, preliminary trials are recommended to assure there are no impacts to phage stability due to site specific water quality characteristics.



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REFERENCES

- Adams, M.H. 1959. Bacteriophages. Interscience; New York.
- Bolton, J.R. and Linden, K.G. 2003. Standardization of Methods for Fluence (UV Dose) Determination in Bench-scale UV Experiments J. Environ. Engng. 129(3): 209-216.
- Hoyer, O. 2000. The Status of UV Technology in Europe. IUVA News 2(1): 22-27.
- NWRI/AwwaRF. 2003. Ultraviolet Disinfection Guidelines for Drinking Water and Water Reuse. 2nd Ed., National Water Research Institute, Fountain Valley, CA.
- Petri, B.M., Gang, F., Malley, J.P., Moran, D.C., and Wright, H. 2000. Groundwater UV Disinfection: Challenges and Solutions. Proc. AWWA Water Qual. Tech. Conf., Salt Lake City, UT. American Water Works Association, Denver, CO.
- USEPA. 2003a. National Primary Drinking water Regulations: Long-Term 2 Enhanced Surface Water Treatment Rule; Proposed Rule. Fed.Reg., 68:154:47640.
- USEPA. 2003b. Ultraviolet Disinfection Guidance Manual (Draft). Office of Water. EPA Report No. 815 D 03-007.

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