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Evaluation of MS-2 Reduction by UV Water Box Water Disinfection

Report

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Project Summary

The Water Box device for disinfection of potable water by UV light was tested for anti-viral efficacy using MS-2 *Escherichia coli* (*E. coli*) bacteriophage. Performance was tested under five different conditions, no UV and 4.1 L of challenge solution, 120VAC power and 4.1 L, hand power and 4.1 L, 120 VAC and 3.1 L, and hand power and 3.1 L. Distilled water was inoculated with approximately $1x10^6$ MS-2 viral particles and contaminated with 1.2 ppm 4-hydroxybenzoic acid (PHBA) to reduce UV transmission to 80 % ± 1 %. A 100 mL sample of contaminated water was collected prior to each test, and in both UV tests the UV was powered for 60 seconds prior to collecting the 100 mL treated samples. Samples were diluted and plated on Tryptic Soy Agar (TSA) plates with *E. coli* inoculum for MS-2 enumeration.

Procedures and Data

UV Transmission Adjustment

The test protocol specified that the challenge water be modified with the addition of PHBA to reduce UV transmission to 80%. An HP spectrophotometer was used to measure the transmission of 254 nm light over a 1 cm path length. Previous testing established that 2 ppm PHBA would reduce transmission to approximately 65 %. Solutions of 1.0, 1.2, 1.4 and 2.0 ppm PHBA in distilled water were used to establish the concentration needed to reduce the transmission to 80 %. Dilutions of a 200 ppm PHBA in distilled water solution were used to prepare each concentration. A 1.0 cm quartz cuvette was filled with each solution and transmission was measured. The table below shows the results these scans.

Concentration PHBA	**254 nm (%T)**
Distilled Water	99.9
1.0 ppm	83.4
1.2 ppm	80.6
1.4 ppm	77.8
2.0 ppm	70.7

The same 200 ppm PHBA stock solution was used to adjust the UV transmission of the challenge solutions. Challenge solution was prepared in 5.0 L aliquots; 30 mL of the stock was added per aliquot, and the transmission of each was measured as described above. The results are below.

Concentration	**254 nm (%T)**
Test 1 Solution	79.6
Test 2 Solution	79.8
Test 3 Solution	79.5
Test 4 Solution	80.8
Test 5 Solution	81.0

Challenge Water Preparation:

The MS-2 inoculum was prepared as in the previous evaluation. Plaque counts of the filtrate determined the viral density was $8.0x10^9$ Plaque Forming Units/mL. This culture was used to inoculate the challenge water. As mentioned above, separate 5.0 L test solutions were prepared for each of the five challenges. In the previous evaluation, the challenge solution was prepared, the three tests were performed, the six samples were diluted, and finally each dilution was plated. This process takes hours to complete, and the time between inoculation, testing, and plating may have contributed to the apparent reduced performance of the test unit. In order to equalize the time from inoculation to testing and testing to plating, each 5.0 L test aliquot was inoculated with 2.0 mL of viral stock just prior to starting the challenge. Also, following UV treatment, the influent and effluent samples were immediately diluted and plated before the next test was performed. The setup and performance of each test was the same, varying only in the UV treatment step and challenge solution volume. To begin, the appropriate volume of challenge water was transferred to the box. Next, a 100 mL "Influent" sample was collected in a sterile bottle via the Water Box outlet. Following the treatment phase, a 100 mL "Effluent" sample was collected at the outlet. The two samples were then diluted and plated on TSA plates. The Water Box was emptied completely and rinsed with de-ionized water between each test.

Test #1:

Test one evaluated how the Water Box materials affected MS-2. The Water Box was filled with 4.1 L of contaminated water, an Influent sample was taken immediately, and then an Effluent sample was taken after 10 minutes.

Test #2:

Test two evaluated MS-2 disinfection when the Water Box UV light was operated with 120VAC power. The Water Box was filled with 4.1 L of contaminated water, an Influent sample was taken immediately, and then an Effluent sample was taken after running the light for 60 s.

Test #3:

Test three evaluated MS-2 disinfection when the Water Box UV light was operated by hand power. The Water Box was filled with 4.1 L of contaminated water, an Influent sample was taken immediately, and then an Effluent sample was taken after running the light for 60 s. The 60 s did not start until the UV light was visible through the view port with steady illumination. This took approximately 4 s.

Test #4:

Test four evaluated MS-2 disinfection when the Water Box UV light was operated with 120VAC power. The Water Box was filled with 3.1 L of contaminated water, an Influent sample was taken immediately, and then an Effluent sample was taken after running the light for 60 s.

Test #5:

Test five evaluated MS-2 disinfection when the Water Box UV light was operated by hand power. The Water Box was filled with 3.1 L of contaminated water, an Influent sample was taken immediately, and then an Effluent sample was taken after running the light for 60 s. The 60 s did not start until the UV light was visible through the view port with steady illumination. As before, this took approximately 4 s.

Results:

Viral plate counts from the five tests are presented in Table 1. The best performance came when using 120 VAC power, while the hand generator consistently yielded poorer disinfection. It appears that preparing the solutions just prior to executing the challenge, then diluting and plating before beginning the next test produced more consistent initial concentrations and higher quality data.

Water Box Test Results

Test #	UV?	Power Source	Test Volume (L)	Initial Viral Load	Post Treatment Viral Load	Log Reduction
1	No	NA	4.1	$2.1x10^6$	$1.8x10^6$	< 0.1
2	Yes	120 VAC	4.1	$8.7x10^5$	$4.1x10^3$	2.3
3	Yes	Hand Gen.	4.1	$2.0x10^6$	$2.7x10^4$	1.9
4	Yes	120 VAC	3.1	$1.6x10^6$	$5.2x10^3$	2.5
5	Yes	Hand Gen.	3.1	$1.2x10^6$	$3.7x10^4$	1.5