THE UNIVERSITY OF ARIZONA
Water & Energy Sustainable Technology Center

Reduction of *Escherichia coli* and MS-2 Coliphage (Virus) by Pontic Technology Thermal Point-of-Use Instrument

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Company: Pontic Technology, LLC
Test Organism (s): *Eschericia coli* (ATCC #25922) and MS-2 coliphage (ATCC #15597-B1)
Test Matrix: De-chlorinated tap water
Temperature(s): Spiked samples tested after 10 minutes flow (~0.26 liters per minute / ~100 galloons per day) at temperatures of 26.3°C (ambient), 40°C, 72°C, 100°C, and 160°C

EXPERIMENTAL DESIGN

Tests were conducted to determine the efficacy of a point-of-use (POU) instrument designed by Pontic Technology for the reduction of *Escherichia coli* and MS-2 coliphage. MS-2 is a non-pathogenic virus that infects *E. coli* and other members of the family *Enterobacteriaceae*. It is commonly used in experiments to determine the efficacy of POU systems because it exhibits first order kinetics from low to higher levels of inactivation. It is also similar in size and shape to many human pathogenic enteric viruses.

Prior to the experiments, 50 liters of tap water were de-chlorinated by the addition of sodium thiosulfate. Physical/chemical parameters such as the temperature, pH, turbidity, electro-conductivity, total dissolved solids, and the chlorine residual were measured and recorded.

A culture of *E. coli* (ATCC #25922) was prepared on the day before testing by inoculating one colony of the test organism into 100 ml of tryptic soy broth (TSB) and incubation overnight at 37°C with agitation to obtain the organisms in the stationary growth phase. On the test date, the bacterial cells were washed by pelleting the cells via centrifugation. The supernatant was discarded and the pellet was re-suspended in 0.01 M phosphate-buffered saline (PBS; pH 7.0). Three washing steps were performed in total to remove the organic matter present in the broth.

Next, small volumes of *E. coli* and MS-2 coliphage (ATCC #15597-B1) stock (previously prepared) were added to a one-liter volume of water from the tank, mixed, and then added back to the tank. The water in the tank was then mixed for one hour using a recirculating pump to ensure complete mixing of the microorganisms throughout the total volume. The final concentration was expected to be approximately 1.0×10^7 colony forming units (CFU)/ml of *E. coli* and 1×10^6 plaque forming units (PFU)/ml of MS-2 virus in the test water. Triplicate influent samples were collected from the tank and assayed to determine the actual bacteria and virus concentrations in the water prior to treatment.

The unit was tested at room temperature (~24°C) with a flow rate of approximately 0.26 liters per minute (~100 gallons per day) using an internal pump in the instrument. Five different temperatures were included in the test, 23.6°C (ambient water temperature), 40°C, 72°C (pasteurization temperature), 100°C (boiling temperature), and 160°C. The samples for bacteria and virus reduction measurements (triplicate samples for each temperature tested) were collected downstream of the unit (i.e., effluent) using a sampling port in which volumes of approximately 150 ml were collected in sterile 250-ml wide-mouth bottles. The samples were collected 10 minutes from the time the instrument reached the target temperature to allow for the residence time of the spiked/inoculated water in the instrument.

From this sample volume, serial 10-fold dilutions were made in sterile PBS. Bacterial assays were conducted on eosin methylene blue (EMB) agar plates. Appropriate dilutions of influent and effluent samples were assayed via the spread plate method on EMB agar plates. In addition, 10-ml and 1-ml samples of each undiluted unit effluent were also assayed via the membrane filtration method on EMB plates. After incubation for 24 hours at 37°C, colonies on each plate were counted, and the levels of CFU recovered per sample determined. The data are reported (see Table 3 below) as the logarithmic reduction using the formula $-\log_{10} (N_{eff} / N_{inf})$, where N_{inf} is the concentration of

E. coli in the influent and N_{eff} is the concentration of *E. coli* in the sample collected after passage through the unit (at various test temperatures). The relationship between log_{10} reduction and percent reduction is illustrated in Table 1 below.

Table 1. Log_{10} Removal versus Percent Reduction.

Log_{10} Removal	**Percent Reduction (%)**
1	90
2	99
3	99.9
4	99.99
5	99.999
6	99.9999
7	99.99999
8	99.999999

For MS-2 virus, 0.1-ml volumes of each dilution were assayed using the double-agar overlay technique with duplicate plates for each dilution. In short, approximately 0.5 ml of a log-phase culture (4 hours growth in tryptic soy broth medium with agitation at 37°C) of host *Escherichia coli* bacterium (ATCC# 15597) were added to 5 ml of molten tryptic soy agar (containing 1% agar) in a test tube. Next, 0.1 ml of each dilution of the test sample was added to separate tubes. In addition, two 5-ml, and two 1-ml samples of each undiluted unit effluent were also assayed via this method. The tubes were then vortexed gently to mix the cultures and poured onto the surfaces of separate tryptic soy agar plates. The plates were swirled gently to cover the entire surface of the plate with the agar overlay. The overlay was then allowed to solidify at room temperature and then the plates were incubated (inverted) for 18 to 24 hours at 37°C. The surviving MS-2 coliphages were enumerated by counting plaques (circular clearings in the bacterial growth on the agar overlays) to determine the number of PFU of virus per milliliter of each sample. The virus log_{10} reduction for each sample was determined using the formula $-log_{10} (N_{eff} / N_{inf})$, where N_{inf} is the concentration of MS-2 in the influent and N_{eff} is the concentration of MS-2 in the sample collected after passage through the unit (at various test temperatures).

RESULTS

The chemical/physical parameters of the de-chlorinated tap water used in the study are shown in Table 2. The results for the reduction/inactivation of *E. coli* and MS-2 coliphage by the Pontic

Technologies thermal point-of-use instrument are shown in Table 3.

Table 2. Characteristics of non-microbiological parameters of test water

Parameter	De-chlorinated Tap H_2O
Chlorine (mg/L)	None
pH	8.24
Temperature (°C)	26.3
Turbidity (NTU)	1.37
Electro-conductivity (µS/cm)	824
Total dissolved solids (TDS) (mg/L)	583

DISCUSSION

Small average reductions of 0.17 $\log_{10}$ and 0.21 $\log_{10}$ were observed for *E. coli* and MS-2, respectively, for effluent samples collected after passage through the instrument with no additional heating. This was likely due to a small amount of heat transfer from the pump.

All of the reductions observed after exposure to temperatures ≥ 40°C were statistically significant ($P \leq 0.05$) for both organisms in comparison to the controls (influent samples). In addition, all of the reductions observed after exposure to temperatures ≥ 72°C were significantly greater for both organisms in comparison to the reductions observed after exposure to 40°C.

The reductions observed in *E. coli* after exposure to 72°C were significantly less than those observed after exposure to 100°C ($P = 0.0008$). Likewise, the reductions observed after exposure to 100°C were significantly less than those observed after exposure to 160°C ($P = 0.00000003$). No *E. coli* were recovered after exposure to 160°C (> 8.30 Log_{10} reduction).

Similarly, the reductions observed in MS-2 after exposure to 72°C were significantly less than those observed after exposure to 100°C ($P = 0.0002$) and the reductions observed after exposure to 100°C were significantly less than those observed after exposure to 160°C ($P = 0.0000005$). As with *E. coli*, no MS-2 were recovered after exposure to 160°C (> 7.77 Log_{10} reduction).

The reductions observed in MS-2 after exposure to 40°C were significantly lower than those observed for *E. coli* ($P = 0.0003$); however the reductions observed for MS-2 after exposure to 72°C and 100°C were significantly greater than those observed for *E. coli* ($P = 0.003$ and $P = 0.00003$, respectively). Therefore, it appears that MS-2 is more temperature sensitive than *E. coli*. The effluent samples for the two organisms collected after exposure to 160°C had reached the detection limit of the assays and thus cannot be compared statistically.

Table 3. Removal of *Escherichia coli* (ATCC #25592) and MS-2 coliphage (ATCC #15597-B1) from de-chlorinated tap water by the Pontic Technologies thermal point-of-use instrument.

Organism	Sample	Log_{10} removal*			
		40°C	72°C	100°C	160°C
Escherichia coli	Sample 1	1.96	4.12	4.51	> 8.30
	Sample 2	2.18	4.22	4.62	> 8.30
	Sample 3	2.27	4.18	4.57	> 8.30
	Average (± SD)	**2.14† ± 0.16**	**4.17† ± 0.05**	**4.57† ± 0.05**	**> 8.30† ± 0.00**
MS-2 coliphage	Sample 1	1.01	4.51	5.65	> 7.77
	Sample 2	1.08	4.73	5.65	> 7.77
	Sample 3	1.07	4.59	5.54	> 7.77
	Average (± SD)	**1.05† ± 0.04**	**4.61† ± 0.11**	**5.61† ± 0.07**	**> 7.77† ± 0.00**

* Initial concentration of *E. coli* was approximately 9.95×10^6 CFU/ml; the initial concentration of MS-2 coliphage was approximately 2.92×10^6 PFU/ml.

SD standard deviation

† Reduction was statistically significant ($P \leq 0.05$) in comparison to the no treatment controls (influent).

> The numbers of bacteria or virus particles recovered were below the detection limit of the assays (< 1 per 20 ml for each). This was equivalent to a > 8.30 Log_{10} reduction for *E. coli* and a > 7.77 Log_{10} reduction for MS-2. The actual reduction could potentially have been greater than this value if either the initial concentration had been higher or if larger effluent volumes had been assayed.