Determination of the efficiency of inactivation of MS2 phage, Poliovirus, *Cryptosporidium parvum* and a bacterial cocktail in Adelaide drinking water– A detailed evaluation

Lazur MC1 UV/US unit

Consultancy performed on site at AWQC.
20th November, 2007
Final Report, 14th January 2008

Australian Water Quality Centre

Table of Contents

1 Scope

This consultancy was conducted by the Australian Water Quality Centre to evaluate the performance of the Lazur MC1 unit. Experiments were performed on-site at AWQC on 20th November 2007 to assess the Lazur MC1 unit, which is a unit representative of the full Lazur / JSC Svarog disinfection units that incorporate both Ultraviolet (UV) and Ultrasonic (US) constituents.

The detailed evaluation of microbial inactivation detailed in this report follows on from a successful pilot experiment performed in June 2007. In this evaluation, the unit was challenged with MS2-phage, Polio virus, infectious *Cryptosporidium parvum*, *E. coli*, *K. pneumoniae*, *L. pneumophila* serogroup 1, and *Ps. aeruginosa*.

1.1 Experimental protocol

1. Approximately 200L of Adelaide mains water was added to a 240L tank
2. The tank was seeded on three separate occasions with 1) MS-2 phage, 2) a bacterial cocktail and poliovirus, and 3) *Cryptosporidium parvum*. The MS2-phage was spiked at an approximate level of 10^{4-6} pfu/mL, the bacterial cocktail at 10^{6-7} cfu/100mL, and the *Cryptosporidium parvum* at 10^4 oocysts/mL.
3. The pump was switched on and the seeded culture allowed to mix at the fully opened position (>18 L/min) for at least 20 minutes.
4. The MC1 UV system was switched on and allowed to warm up for at least 10 minutes.
5. Sample taps (inlet and outlet) were flamed according to AWQC protocol WMZ-500.
6. The seeded water was throttled through the UV unit and the flow rate adjusted to the desired test flow rate, and the US system turned on. The flow was allowed to stabilise before sampling (90 sec for 6L/min, 60 sec for 12 L/min, and 30 sec for 18 L/min)
7. Pre and post-UV/US samples were taken for each flow rate with both the UV and US on
8. A sample for UVT was taken at the start and completion of the experiment.
9. In between runs, the tank and MC1 unit were disinfected with Chlorine (approx 20ppm for 5 min contact time), flushed, neutralised with excess sodium thiosulphate, and flushed twice again before refilling the tank with test water and seeded as required.

Although flow rates were pre-set by the manufacturer, the flow rates were determined to be 4.5, 12, and 20 L/min as determined by AWQC personnel. Therefore, these flow rates will be reported on.

Prior to the testing described here, the rig was thoroughly disinfected and total bacterial counts from source water and water exiting the unit (without UV and US on) were <100 cfu/mL as determined by standard plate count techniques.

1.2 Collimated Beam Testing

UV dose response data for MS2 phage were prepared in order to correlate the measured log inactivation of phage with UV dosage. This was done with a collimated beam apparatus using the test water. The same batch of phage for the collimated beam testing and test rig experiment were used on both occasions. UV dose in the test rig was then calculated by correlation of log inactivation obtained in the test rig with data from the standard curve in the collimated beam apparatus. Data for the collimated beam apparatus testing was collected at fluences of 0, 10, 20, 40, 60 and 80 mJ/cm^2.

1.3 Analyses

Counts for MS2 phage by plaque assay were performed according to the methodology described in Appendix D of the Draft USEPA UV Disinfection Guidance manual (USEPA (2006) UV Disinfection Guidance Manual, United States Environmental Protection Agency)

Counts for *E. coli*, *K. pneumoniae*, *Ps. aeruginosa*, and *L. pneumophila* were determined using AWQC standard protocols (based on Australian Standards).

Cryptosporidium inactivation was determined by firstly examining oocyst integrity post UV and US using flow cytometric methods, and secondly by cell culture-PCR infectivity assays developed by the AWQC (Keegan, A.R., Fanok, S., Monis, P.T., Saint, C.P., 2003. Cell culture-Taqman PCR assay for evaluation of Cryptosporidium parvum disinfection. *Appl Environ Microbiol* **69**, 2505-2511.

Inactivation of Polivirus was determined by standard cell culture assays and $TCID_{50}$

2 Experimental results

2.1 Run 1 –Collimated Beam Testing and MS2 inactivation

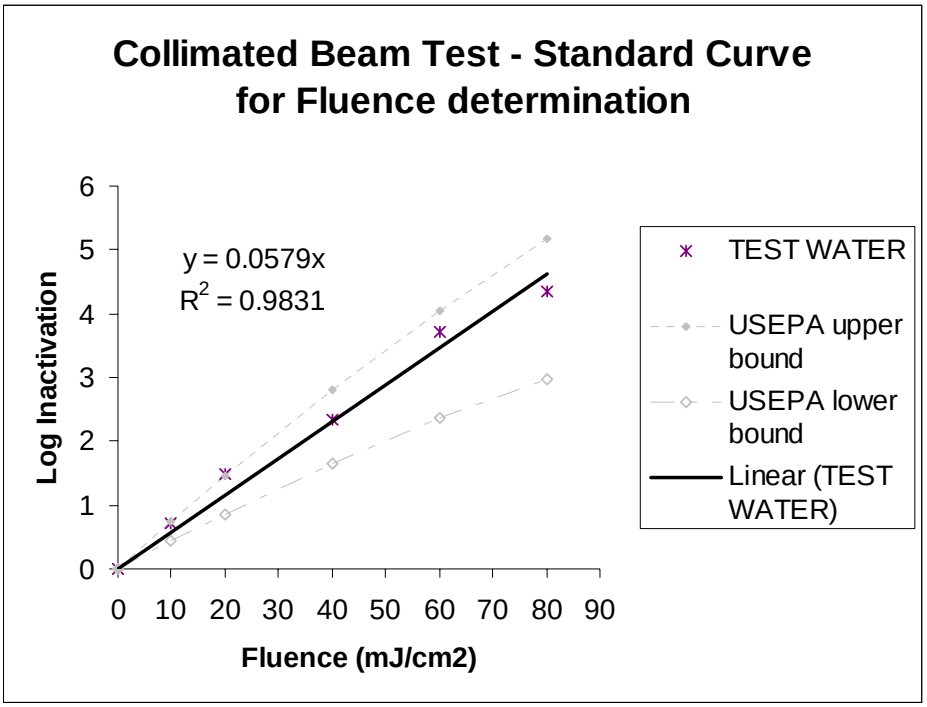

Figure 1. Collimated beam dose response data graph

Table 1. Inactivation of MS2 by Lazur MC1

UV+US		
Flow rate (L/min)	**MS2 Log Inactivation**	**Equivalent Fluence* (mJ/cm^2)**
4.5	5.43	>75.3
12	5.03	>75.3
20	3.78	65.2

* NOTE: The observed log inactivation of MS2 has been expressed as an equivalent fluence dose (i.e. the dose that would cause inactivation by UV alone). If US has no effect on inactivation of MS2, then the equivalent UV dose is the actual UV dose delivered. The effect of US on MS2 has not been determined in this study. This is a deviation from the USEPA guidelines and results should be interpreted accordingly.

2.2 Run 2 – E. coli, K. pneumoniae, Ps. aeruginosa, L. pneumophila serogroup1, and Polio virus

	***E. coli* PRE**				***E. coli* POST**				**Log inactivation**
Flow rate (L / min)	replicate 1	replicate 2	replicate 3	average	replicate 1	replicate 2	replicate 3	average	
4.5	8.10E+07	8.10E+07	9.10E+07	8.43E+07	<1	<1	1.00E+00	1.00E+00	7.9
12	6.70E+07	1.00E+08	1.00E+08	8.90E+07	<1	<1	<1	<1	>7.9
20	6.10E+08	7.90E+08	7.00E+08	7.00E+08	<1	<1	<1	<1	>8.8

	***Ps. aeruginosa* PRE**				***Ps. aeruginosa* POST**				**Log inactivation**
Flow rate (L / min)	replicate 1	replicate 2	replicate 3	average	replicate 1	replicate 2	replicate 3	average	
4.5	3.40E+08	2.10E+08	3.30E+08	2.93E+08	<1	<1	<1	<1	>8.4
12	3.60E+08	4.00E+08	4.00E+08	3.87E+08	<1	<1	<1	<1	>8.5
20	3.20E+08	4.30E+08	4.10E+08	3.87E+08	<1	<1	3.00E+00	3.00E+00	8.1

	***L. pneumophila* PRE**				***L. pneumophila* POST**				**Log inactivation**
Flow rate (L / min)	replicate 1	replicate 2	replicate 3	average	replicate 1	replicate 2	replicate 3	average	
4.5	3.30E+03	3.15E+03	2.00E+03	2.82E+03	<10	<10	<10	<10	>2.4
12	3.30E+03	1.00E+03	8.00E+02	1.70E+03	<10	<10	<10	<10	>2.2
20	2.40E+03	2.95E+03	1.20E+03	2.18E+03	<10	<10	<10	<10	>2.3

	Polio PRE				**Polio POST**				**Log inactivation**
Flow rate (L / min)	replicate 1	replicate 2	replicate 3	average	replicate 1	replicate 2	replicate 3	average	
4.5	2.24E+04	3.14E+04	2.54E+04	2.64E+04	<100	<100	<100	<100	>2.4
12	2.92E+05	1.66E+05	2.11E+05	2.23E+05	<100	<100	<100	<100	>3.3
20	1.14E+05	1.03E+05	4.70E+04	8.80E+04	<100	<100	<100	<100	>2.9

Notes:

1. Ultraviolet transmission (UVT) of water was 73%.
2. *K. pneumoniae* counts were not obtainable for the dilutions analysed.
3. *E. coli* and *Ps. aeruginosa* results in cfu/100mL. *L. pneumophila* and Polio virus results in cfu/mL.
4. "<" indicates that organism wasn't detected and fell below the detection threshold of the analyses

2.3 Run 3 – Cryptosporidium inactivation

Test water UVT was 83%

Oocyst integrity and number were assessed by flow cytometry dot plots (Figure 2). This figure shows that the oocyst structure is not affected by UV and US. This determination was important as it demonstrates that any inactivation of *Cryptosporidium parvum* was not due to any processes that compromised oocyst integrity.

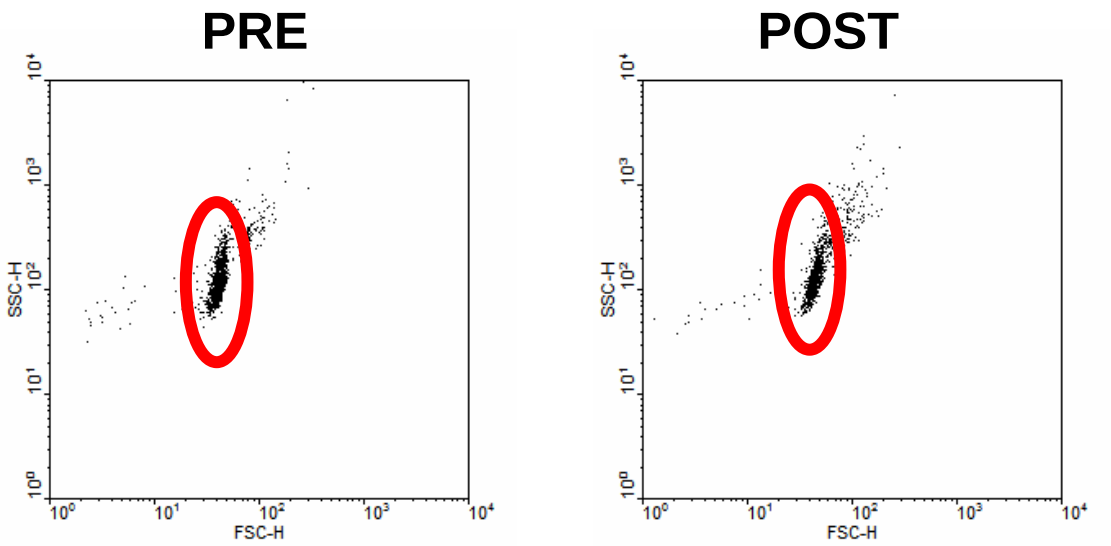

Figure 2. Flow cytometry dot plot of *Cryptosporidium parvum* through the MC1 unit, PRE and POST UV+US

Average counts for PRE were 12600 oocysts/mL, and POST 11800 oocysts/mL across all the flow rates. Detailed investigations at individual flow rates indicated no significant differences ($p > 0.05$) between the PRE and POST oocyst numbers.

Infectivity of oocysts were determined by cell culture-PCR assay as described earlier. The results are presented in the Table 3

Table 3. *Cryptosporidium parvum* inactivation

Flow rate (L/min)	Log Reduction in Infectivity
4.5	>3.88
12	>3.88
20	>3.88

3 Conclusions

- Phage inactivation at the highest flow rate (20 L/min) was equivalent to a dose of 62 mJ/cm^2, and at 4.5 and 12 L/min the unit delivers a greater equivalent dose

- The unit effectively removed greater than 7.9 to 8.8–log of *E. coli* , and *Ps. aeruginosa* in drinking water with a UVT of 73%

- The unit demonstrated >3.88 log inactivation of *Cryptosporidium parvum* in drinking water with a UVT of 83%

- The unit effectively inactivated all spiked *L. pnuemophila*, *Cryptosporidium parvum*, and Polio virus at all flow rates, with only a minimal amount of *E. coli* and *Ps. aeruginosa* detected at 4.5 L/s and 20 L/s respectively (under these test conditions).