Microbial challenge-testing of treatment processes for quantifying stormwater recycling risks and management


ABSTRACT

Pathogenic microorganisms have been identified as the main human health risks associated with the reuse of treated urban stormwater (runoff from paved and unpaved urban areas). As part of the Smart Water initiative (Victorian Government, Australia), a collaborative evaluation of three existing integrated stormwater recycling systems, and the risks involved in non-potable reuse of treated urban stormwater is being undertaken. Three stormwater recycling systems were selected at urban locations to provide a range of barriers including biofiltration, storage tanks, UV disinfection, a constructed wetland, and retention ponds. Recycled water from each of the systems is used for open space irrigation. In order to adequately undertake exposure assessments, it was necessary to quantify the efficacy of key barriers in each exposure pathway. Given that none of the selected treatment systems had previously been evaluated for their treatment efficiency, experimental work was carried out comprising dry and wet weather monitoring of each system (for a period of 12 months), as well as challenging the barriers with model microbes (for viruses, bacteria and parasitic protozoa) to provide input data for use in Quantitative Microbial Risk Assessment.

Key words | pathogens, reuse, stormwater

INTRODUCTION

Urban stormwater carries significant quantities of debris and pollutants that include litter, oils, heavy metals, sediment, nutrients, organic matter, and microorganisms. The quantity and range of microbial pollutants carried by stormwater are influenced by the degree of contamination from inputs such as septic tank seepage, sewer leakage and overflow, and domestic animal faeces (Davies & Bavor 2000). Epidemiological evidence has suggested that there is an increased risk of adverse health associated with recreational contact with untreated urban stormwater (Haile et al. 1999; Colford et al. 2007). In addition, public perception of health risk associated with microbiological contamination of water remains one of the main impediments to water recycling and reuse (Higgins et al. 2002). It is imperative, therefore, that urban stormwater is treated adequately prior to reuse.

In order to adequately assess health risk exposure, it is necessary to quantify the effectiveness of different barriers in each exposure pathway. Purposely challenging treatment processes with non-pathogenic model or surrogate microorganisms offers a viable means by which to assess potential pathogen reduction efficiency and identify system weaknesses that would otherwise go undetected if relying on environmental indicators or pilot-scale studies (Signor et al. unpublished).

Permissions from relevant health and government authorities were obtained to conduct short-term challenge testing of the three stormwater recycling systems with the proposed model surrogate microorganisms to represent viral, bacterial, and protozoan pathogens, with the objective of generating input data for use in a Quantitative Microbial Risk Assessment (QMRA) of each system.

doi: 10.2166/wst.2008.194
METHODS

Surrogates and microbiological analysis

Bacteriophage MS2 (ATCC 15597-B1) stock suspensions for spiking were prepared by infecting host cells of *Salmonella typhimurium* strain WG49 (NCTC 12484). MS2 concentrations in the challenge test samples were assayed by the double agar layer technique (Adams 1959; ISO 1995) using WG49 as the host.

*E. coli* (ATCC 13706) used for system spiking was grown up from frozen stocks (−80°C) overnight in 100 mL of Tryptone Yeast Extract Glucose Broth at 37°C. Most probable number concentrations of *E. coli* in the challenge test samples were determined using Colilert-18™ in the Quantitray 2000® format (Idexx Laboratories Inc., Rydalmere, NSW, Australia). *E. coli* ATCC 13706 is not a host for MS2 since it does not possess the F pili.

Food-grade baker’s cream yeast (*Saccharomyces cerevisiae*) was obtained from an ISO 9001 accredited supplier. Yeast concentrations in the challenge test samples were determined by the plate count technique using Dichloran Rose Bengal Agar incubated at 20°C for 5 days, with confirmation by microscopy.

Study sites

Three stormwater treatment systems were selected for study. The key features of each system are illustrated in Figure 1.

The barriers that are shaded in Figure 1 indicate that challenge testing with microbial surrogates was undertaken on this treatment barrier. Water restrictions have prevented further challenge tests being carried out to date.

Biofilter and sedimentation tank challenge tests

Each of the microorganisms used to seed the feedwater were transported to the site separately. A spiking cocktail was prepared on site immediately prior to performing the challenge test by the addition and mixing of the microorganisms into 30 L of tap water with resulting approximate expected total numbers (in 30 L) as follows:

- **MS2** $10^{11}$
- **E. coli** $10^9 - 10^{10}$
- **Yeast** $10^{11}$

Monash sedimentation tanks and biofilter were challenged in duplicate 24 hours apart. Pond water was pumped into the treatment units to simulate the inflow of stormwater. The surrogate microorganisms were then introduced into the inflow from the 30L cocktail. For the biofilter, a separate pump was used to pump the cocktail into the inflow. The inflow was then split between two cells of the three cells, each containing different filter media: vermiculite (biofilter A) and mulch (biofilter B), representing the best and worst cells, respectively, in terms of pollutant removal efficiency (based on previous observations for removal of nutrients). In the case of the sedimentation tanks, the cocktail was introduced into the inflow by manually pouring it in at an approximate constant rate.

Unspiked pond water was collected immediately prior to each challenge test to be analysed for background concentrations of the three surrogate microorganisms: MS2, *E. coli*, and yeast. Five influent samples were collected in 500 mL sterile containers by manual operation of an autosampler. The samples were collected at 15 second intervals for the biofilter challenge tests and at 30 second intervals for the sedimentation tank challenge tests, with flushing of the autosampler lines between each sample being collected. Inflow of pond water was maintained beyond this time for several minutes. A blank sample of deionised water (500 mL), and a 10 mL subsample of each spiking cocktail were also collected.
Ten effluent samples were collected in 500 mL containers by autosamplers that had been preset to collect samples according to flow.

**UV lamp challenge tests**

To ensure adequate mixing of the spiked microorganisms into the feedwater, the spiking point was located greater than 10 diameter-lengths upstream of the influent sampling point. Subsequent additional mixing between the influent sampling point and the reactor was also expected to occur due to the presence of two 90° bends in the feed pipe just prior to the UV reactor. The effluent sampling point was installed at least 5 pipe diameters downstream of the reactor.

A pressure test bucket pump (Rigid 1450, Ridge Tool Company, Emerson, St. Louis, Missouri, USA) was used for pumping the surrogate microorganisms (in this test only MS2) into the recycled water stream at the spiking point prior to the UV. Ten samples of influent and ten samples of effluent were collected at 15 second intervals during the 2 minute period. A blank sample of deionised water (500 mL), and a 10 mL subsample of the MS2 spiking suspension were also collected.

A collimated beam apparatus was designed and set up. In order to minimise the inherent variation associated with collimated beam tests the apparatus was designed and operated in compliance with the quality control and quality assurance considerations specified by USEPA (2006) and NWRI-AwwaRF (2000). Suspensions of MS2 were used to spike 30 mL portions of Royal Park feedwater that had been collected immediately prior to the challenge test. The suspensions were irradiated in a sterile Petri dish (90 mm diameter, with lid removed) at room temperature, in duplicate at the low-pressure UV lamp wavelength (254 nm).

The arithmetic mean and standard deviation (SD) of the log₁₀ influent and effluent challenge microorganism concentrations were determined for each flowrate. Log inactivation was calculated using:

\[
\text{Log inactivation} = \log_{10}(N_o/N)
\]

where, \(N_o\) is the influent concentration and \(N\) is the effluent concentration.

The UV dose applied at 254 nm during the collimated beam tests was calculated using:

\[
D = E_s P_f (1 - R) \frac{L (1 - 10^{-ad})}{(d + L) \times \text{ad} \ln(10) t}
\]

where, \(D\) was the UV dose in mJ.cm⁻², \(E_s\) was the irradiance at the centre of the suspension surface (mW cm⁻² mean of before and after measurements by the radiometer), \(P_f\) was the Petri factor, \(R\) was the reflectance at the air-water interface at 254 nm, \(L\) was the distance from lamp to suspension surface (cm), \(d\) was the depth of suspension (cm), \(a\) was the UV absorption coefficient of the suspension at 254 nm (cm⁻¹), and \(t\) was the exposure time (seconds) (USEPA 2006).

**RESULTS AND DISCUSSION**

**Royal park UV system**

According to the relationship determined by the collimated beam experiments, and shown in Figure 2, a 0.3-log reduction of MS2 suggests that during the challenge test the bacteriophages received a dose of somewhere between 0 and 8.9 mJ.cm⁻². Linear interpolation indicates that a dose of 5.2 mJ.cm⁻² was received by the MS2.

Assuming that other microorganisms passing through the UV reactor would also receive a dose of 5.2 mJ.cm², but taking into account that different microorganisms have
different sensitivities to UV, the estimated UV inactivation of various pathogens were estimated, and are shown in Table 1.

Monash biofilter and sedimentation tanks

The background concentrations of MS2, *E. coli* and yeast in inflowing pond water were 2–6 orders of magnitude lower than concentrations of these microorganisms detected in the spiked influent samples. None of the blank samples analysed contained any of the three test microorganisms in the volumes analysed.

Table 2 shows the measured log reductions for each of the surrogate microorganisms in the biofilter as determined using the mean concentrations in versus mean concentrations out, and the variability in brackets shows the range of log reductions if each effluent concentration was considered separately in comparison to the influent. The log reduction values given in Tables 2 and 3 for *E. coli*, MS2, and yeast might be expected to be similar to values expected generally for bacterial, viral and protozoan pathogens, respectively.

Overall reduction of microbial surrogates by the Monash biofilter was highest for the yeast, followed by *E. coli* and then viruses, corresponding to the relative organism size from largest to smallest. This initial data analysis is only a comparison of inflow and outflow concentrations and does not consider the overall pollutant response of the biofilter. In reality, the short-term initial loading of the filter will produce a distribution of concentration at the outflow which will vary with time as the pollutant travels through the filter media.

Table 3 shows the log reductions for each of the microorganisms in the sedimentation tanks determined using the mean concentrations in versus mean concentrations out, and the variability in brackets shows the range of log reductions if each effluent concentration was considered separately in comparison to the influent.

There appeared to be a discrepancy between the duplicate sedimentation tank challenge tests, with the first test showing greater reductions in surrogate organisms. The replicate tests were conducted 24 hours apart, and, therefore, the lower reductions for the second test may be due to resuspension of residual organisms contained with sediment from the first test. Overall, the reduction appeared greatest for MS2 bacteriophage, followed by *E. coli* and yeast. Despite their smaller size, virus removal may be greatest due to their greater affinity for particulates.

For the challenge tests food-grade baker’s yeast (*Saccharomyces cerevisiae*), and the bacteriophage MS2 were selected as surrogates of the protozoan pathogen *Cryptosporidium parvum* and of human enteric viruses, respectively. Common baker’s yeast (*Saccharomyces cerevisiae*) is a ‘generally regarded as safe’ organism found abundantly in many foods and beverages (USEPA 1997). Chung et al. (2004) demonstrated that *S. cerevisiae* responds to physical water treatments in a manner that is similar to oocysts of the pathogenic protozoan *Cryptosporidium parvum*, and was considered an appropriate surrogate for protozoan trans-

<table>
<thead>
<tr>
<th>Reference pathogen</th>
<th>Estimated Log10 Reduction at UV dose of 5.2 mj.cm⁻²</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Campylobacter jejuni</em></td>
<td>2.69</td>
</tr>
<tr>
<td><em>Salmonella enterica</em></td>
<td>4.59</td>
</tr>
<tr>
<td>Adenovirus 2</td>
<td>0.125</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>0.532</td>
</tr>
<tr>
<td><em>Cryptosporidium parvum</em></td>
<td>2.26</td>
</tr>
</tbody>
</table>

*Using inactivation data from Hijnen et al. (2006).*

Table 2 | Log₁₀ reductions of pathogen surrogates by the Monash biofilter treating stormwater

<table>
<thead>
<tr>
<th>Biofilter cell</th>
<th>Replicate challenge test</th>
<th>Log₁₀ Reductions [Minimum and Maximum] Yeast</th>
<th>Bacteriophage MS2</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>1.77 [1.25–3.06]</td>
<td>2.95 [2.63–3.93]</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.59 [1.12–3.30]</td>
<td>2.30 [1.92–3.48]</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>1.76 [1.56–2.20]</td>
<td>1.69 [0.84–2.3]</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.40 [1.06–2.27]</td>
<td>2.23 [1.92–3.32]</td>
</tr>
</tbody>
</table>

Table 1 | Estimated UV inactivation of various pathogens based on measured UV inactivation of MS2 bacteriophage in Royal Park stormwater

<table>
<thead>
<tr>
<th>Reference pathogen</th>
<th>Estimated Log₁₀ Reduction at UV dose of 5.2 mj.cm⁻²</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Campylobacter jejuni</em></td>
<td>2.69</td>
</tr>
<tr>
<td><em>Salmonella enterica</em></td>
<td>4.59</td>
</tr>
<tr>
<td>Adenovirus 2</td>
<td>0.125</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>0.532</td>
</tr>
<tr>
<td><em>Cryptosporidium parvum</em></td>
<td>2.26</td>
</tr>
</tbody>
</table>
port. MS2 has long been used as a model microorganism for studying the movement of viruses in the environment, and is well established as a surrogate for assessing the efficiency of UV disinfection systems (USEPA 2006). 

(E. coli) was included as a model for bacterial pathogens and to provide a link to the microbiological monitoring data collected in the study.

Challenge testing with microbial surrogates was useful for evaluating the performance of treatment barriers under site specific conditions and provides basic information for microbial risk assessment and verification of full-scale system components—both necessary components when undertaking a water safety plan approach to managing safe water (NWQMS 2006). The data provided by conducting the challenge tests, along with data from the literature, will be used in a screening-level quantitative assessment of the human health risk associated with non-potable stormwater harvesting at each of the systems.

### References


