Removal of *Escherichia coli* in Stormwater Biofilters

K.K.G.I. Chandasena[1]*, A. Deletic[1], J. Ellerton[1] and D.T. McCarthy[1]

[1] *Centre for Water Sensitive Cities, Department of Civil Engineering, Building 60, Monash University, VIC 3800, Australia*

**Corresponding author, e-mail Gayani.Chandrasena@monash.edu*

ABSTRACT

Biofilters are common, low energy technologies used for the treatment of urban stormwater. They have shown promising results in removal of stormwater microorganisms, but the factors affecting the reported removal need to be investigated. Hence, this study investigated the effects of particle-microbial interaction, inflow concentration, background microbial concentration and plant species on microbial removal capacity. The experimental methods consisted of a biofilter column study to evaluate removal performance and a sequential filtration procedure to estimate microbial partitioning. Columns were dosed with different concentrations of free phase *E. coli* only and *E. coli* mixed with stormwater sediment. The results indicate that the microbial removal is significantly affected by inflow concentration and background microbial levels. Outflow concentration increased with increasing inflow concentration for both applications. Leaching was observed when a low concentration inflow event occurred after a very high inflow concentration event. *Lomandra longifolia* showed better removal compared to *Carex appressa.*

KEYWORDS

Biofilters, *Escherichia coli*, stormwater, removal performance

INTRODUCTION

It is well documented that urban stormwater is contaminated with a number of pollutants and therefore needs to be treated before it can be safely harvested (Struck *et al.*, 2006). Stormwater biofilters are a low energy treatment technology established under Water Sensitive Urban Design (FAWB, 2009). The capacity of these biofilters in removing various stormwater pollutants which impact ecosystem health, such as sediment, heavy metals and nutrients (Hatt et al., 2009, Bratieres et al., 2008b) have been researched to a greater extend compared to other pollutants which adversely impact human health, especially human pathogens (Bratieres et al., 2008a, Rusciano and Obropta, 2007). The few studies which have evaluated the microorganism removal capacity of biofilters have shown a wide range of performances, from net export to very high removal. For example, Hathaway *et al.* (2009) has evaluated two field bioretention systems in North Carolina and reported percentage removals ranging from -611% to 92% for *E. coli*, -132% to 86% for *Enterococci* and 89% for faecal coliforms. This variance in removal capacity was hypothesized to be affected by design parameters such as media depth and composition.

Generally, microorganisms will be removed by biofiltration systems through straining, adsorption, inactivation due to temperature and moisture, predation and competition (Rusciano and Obropta, 2007, Stevik *et al.*, 2004, Zhang *et al.*, 2010). However, the relative contribution of each process is still poorly understood. In respect to the contribution made to microorganism removal due to straining, it is well documented that stormwater microorganisms are associated

to particles present in stormwater (Schillinger and Gannon, 1985, Characklis *et al.*, 2005) which significantly impacts straining (Ferguson *et al.*, 2003). However, little work has been conducted on determining this contribution for stormwater biofilters. Furthermore, the role of biofilter plants in pathogen removal is often overlooked. Rusciano and Obropta(2007) stated that creation of macrospores near to roots may interfere with the removal performance. On the other hand, it is reported that microorganisms colonize in the root zone or the rhizoshpere of plants and the some root exudates are reported to have contain antibiotic substances (Brix, 1997). As such, plant's root system may important in pathogen removal.

Consequently, the present study investigates several factors important in pathogen removal in stormwater biofilter. Firstly, the effect of suspended particle-microorganism interactions on removal processes by biofilters will be studied using a set of laboratory based biofilter columns. These will be dosed with free phase *E. coli* and *E. coli* mixed with sediment. The effect of inflow concentration and background microorganism level in microbial removal will be studied using a range of inflow concentrations. All of these experiments will be carried out using two common biofilter plants species and hence it will provide some insight into the effect of plants in removal performance.

METHODS

To investigate the abovementioned aims, two laboratory experiments were conducted: (1) dosing of laboratory scale biofiltration systems to assess the microbial removal in biofiltration systems and (2) sequential filtration tests to assess the association of *E. coli* to particulate matter.

Laboratory experiments to assess microbial removal in biofiltration systems

Biofilter columns were used to study the microbial removal performance using *Escherichia coli* (*E. coli*) as the model microorganism. Although there have been many publications regarding the disadvantages of using *E. coli* as a model microorganism (Ishii *et al.*, 2006), it was still selected in this study as it is being used as an indicator of faecal contamination in many guidelines around the world, including current Australian Drinking Water and Stormwater Harvesting guidelines.

Column configuration. 25 square (300x300x600mm deep) biofilter columns were used, all of which had well established vegetation (>15 months old). Columns were filled with loamy sand media in accordance with adoption guidelines for stormwater biofltration systems (FAWB, 2009) and the recommended particle size percentages are shown in Table 1. Filter media consisted of three layers namely loamy sand (400 mm), transition layer of washed sand (100 mm) and a drainage layer of gravel (100 mm) containing a collection pipe. Columns were planted with a mix of 4 plants; 4 *Carex appressa* (C4/L0), 3 *C. appressa* and 1 *Lomandra longifolia* (C3/L1), 2 C. appressa and 2 *Lomandra longifolia* (C2/L1), 1 *C. appressa* and 3 *Lomandra longifolia* (C1/L3), and 4 *Lomandra longifolia* (C0/L1). All configurations had five replicates.

Table 1. Recommended proportion of the soil particles comprising the filter media.

Particle type	Size (mm)	Proportion (w/w%)
Clay and silt	< 0.05	< 3
Very fine sand	0.05 – 0.15	5 – 30
Fine sand	0.15 – 0.25	10 – 30
Medium to coarse sand	0.25 – 1.0	40 – 60
Coarse sand	1.0 – 2.0	7 – 10
Fine gravel	2.0 – 3.4	< 3

Source: (FAWB, 2009)

Dosing. To test the influence of inflow concentrations on removal capacities, the biofilter columns were dosed with different concentrations of *E. coli*. Also, to determine the influence of *E. coli* particle association on treatment, the biofilters were dosed with two different inflow mixtures: (1) semi-natural stormwater ("*E. coli* + sediment") or (2) free-phase *E. coli* ("*E. coli* only"). To create the '*E. coli* only' mixture, *E. coli* culture (ATCC#11775) was dosed into 500L of dechlorinated tap water (die-off of these organisms in this tap water has been shown to be insignificant over the testing period). To create the '*E. coli* + sediment' mixture, sediment collected from a local stormwater retarding pond was sieved through a 1 mm screen was added to de-chlorinated tap water to match a target TSS concentration of 150 mg/L (Wong, 2006). *E. coli* was then dosed into the mixture to achieve the target concentration. Semi-natural stormwater was used in this study mainly because it was not possible to collect a large volume of natural stormwater for each dosing day and it was quite easy to maintain the consistency of inflow using semi-natural stormwater. The first application of '*E. coli* + sediment' dosing utilized only 10minutes mixing in a large tank and this was extended in subsequent dosing to allow for increased contact time between the *E. coli* and sediment. More specifically, to test the influence of mixing time on association between *E. coli* and particles, the total amount of sediment and *E. coli* required for the dosing was first added to just 8L of tap water and mixed at 20rpm for 24hr prior to dosing on the 23rd May and 4th April dosing events and mixed for 3hours prior to the 6th April dosing event (see Table 2 for more info). Inflow mixtures were prepared in a 5000 L 'Colourbond' water tank with an internal agitator driven by a 1.5 kW electric motor and rotating at 200rpm to keep the sediment in suspension. Columns were designed to be dosed with 12.6 L, three times a week to mimic effective mean annual Melbourne rainfall. An automated delivery system was used to supply inflow solution at a rate of 190 L/min which maintains the TSS and provides a consistent supply of water to each column.

Table 2. Inflow characteristics in different dosing events

Date	Event number	Type	Concentration (MPN/100mL)
02/03/2011	Run 1	*E. coli* only	8.66×10^{4}
04/03/2011	Run 2	*E. coli* only	1.79×10^{3}
07/03/2011	Run 3	*E. coli* only	1.17×10^{3}
15/03/2011	Run 4	*E. coli* + sediment	8.84×10^{2}
23/03/2011	Run 5	*E. coli* + sediment	2.01×10^{4}
04/04/2011	Run 6	*E. coli* + sediment	6.25×10^{5}
06/04/2011	Run 7	*E. coli* + sediment	1.36×10^{3}
08/04/2011	Run 8	*E. coli* only	1.36×10^{2}

Sampling. Composite samples were collected from the inflow and the column outlets. The sampling regime was designed to collect 50mL after the first 1L of outflow and then three more 50mL sub-samples after every 3Ls of outflow. Furthermore, all outflow samples were collected within three hours of taking the first 50mL sub-sample; this was to avoid long sample holding times prior to analysis. As such, while this composite sampling procedure was followed during most of the sampling events, final sub-samples for some of the columns had not been taken even after 3hrs, in which case the final sub-sample was collected regardless of the cumulative volume of outflow. As such, recorded cumulative outflow volumes passed during the 3hr period varied between, and within, different configurations. However, there was no direct correlation between cumulative outflow volume and removal percentage. *E. coli* concentrations in all samples were analysed using the Colilert™ method (IDEXX-Laboratories, 2007). It should be noted that in cases where the outflow concentration was lower than the detectable limit (1MPN/100mL), half of the lowest detectable limit was taken as the concentration for statistical analysis (plotting and table summary statistics).

Assessment of *E. coli* association to particles

Inflow samples consisting of '*E. coli* + sediment' were analysed using sequential filtration in triplicates. Inflow samples were passed through series of 47mm diameter Nylon filters of pore size 100, 60, 30, 10, 5 and 3μm, respectively. Preliminary filtration tests carried out using free-phase *E. coli* have shown that the percentage free-phase *E. coli* attached to filters is minimal. Furthermore, flow rate through the sequential filtration set up was maintained similar to those flow rates that occur in typical biofilters to minimise possible disturbance of particle association characteristics due to application of high pressure. After filtration, material retained on each filter was resuspended in deionised water containing 0.02% Tween 80 and agitated at 20rpm for 10 minutes. Thereafter, aliquots taken from solutions corresponding to each filter and the filtrate were analysed for *E. coli* using the Colilert method. Furthermore, the results obtained from this study were compared against observed microbial partitioning characteristics of an existing biofilter inlet (Banyan Reserve, Melbourne) which was assessed using same sequential filtration method.

RESULTS AND DISCUSSION

Microbial particle association

The results of sequential filtration test, blank test and data collected from the inlet of an existing biofilter (Banyan Reserve, Melbourne) are shown in Figure 1. It is evident that any of the mixing methods tested have not been able to produce significant partitioning and hence, the majority of the *E. coli* (93.84 ±6%) remain in the free phase or attached to smaller particles. However, the achieved partitioning is comparable with observed particle association of *E. coli* in a biofilter treating stromwater from a small residential catchment in Melbourne. Furthermore, Davies and Bavor (2000) found that stromwater bacteria are associated with small particles (<2μm). These findings suggest that the approach used here to test biofilters (i.e. spiking with *E. coli*) is somewhat representative of field conditions. Furthermore, these findings suggest that straining will not play a significant role in biofilters (both in laboratory and field conditions) in removing *E. coli*, as almost all of the *E. coli* are able to pass through the 3μm filter. Comparing this with the pore sizes of the biofilter (Table 1, only 3% of the media is <50μm) suggests that *E. coli* are not being strained within the media (see Stevik *et al*., 2004) for more information on the relationship between straining efficacy of bacteria and filter media particle size distribution).

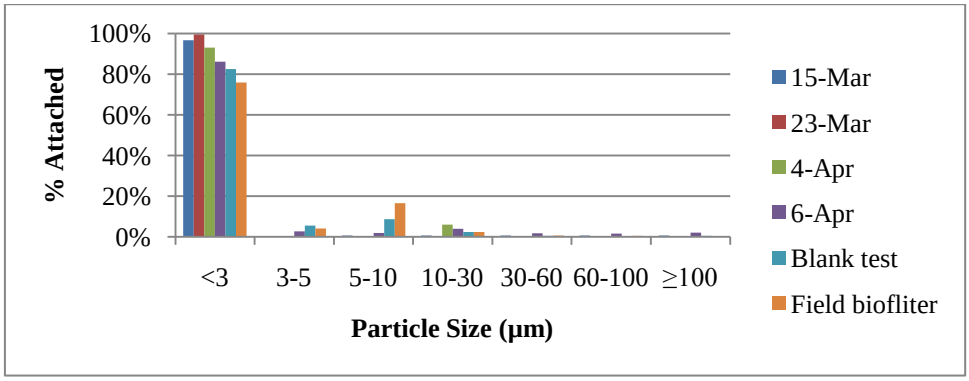

Figure 1. Percentage *E. coli* attached to different particle sizes

Removal performance

Figure 2 summarizes the average removal performance over the full period of dosing for the configurations which contained four *Carex appressa* plants, while Figure 3 shows the mean outflow concentrations from each of the configurations as a function of the inflow concentrations. In general, there was a high variability in the removal performance of the biofilters, with higher log reductions achieved when using the '*E. coli* + sediment' application method. However, against initial hypotheses, and as discussed above, this higher removal performance was not caused by improved straining efficiency. It is possible that there are other characteristics of the "*E. coli* + sediment" application method which could result in the observed differences (e.g. sediment is causing some other influences such as predation or competition). It is however hypothesised that the differences seen in the results are most dependent on the inflow concentrations of *E. coli* (irrespective of the application type) and the antecedent conditions experienced by the biofilters. This hypothesis is discussed in detail below, together with a discussion about how the results reinforce this hypothesis.

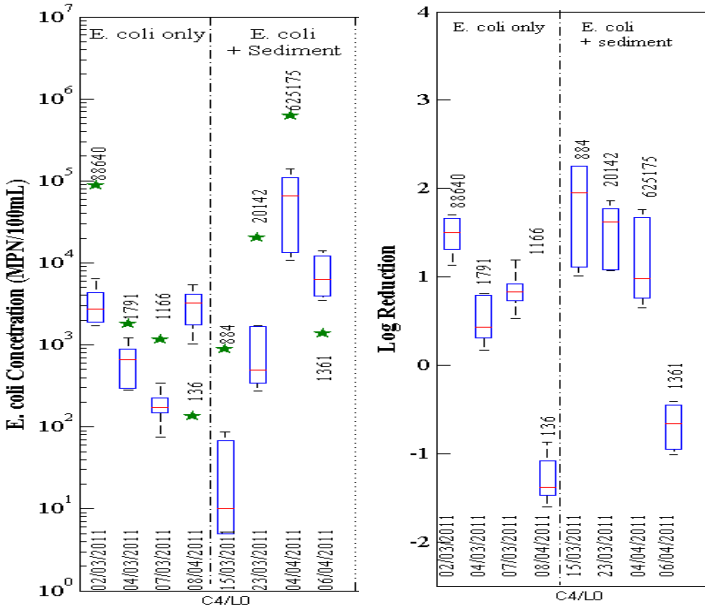

Figure 2. Removal performances for E. coli only and 'E. coli + sediment' (a) outflow concentration (MPN/100mL) (b) log reduction. An example is only provided for the configuration which had 4 Carex appressa plants

It is hypothesized that microorganisms are mainly removed by the filter through adsorption processes, but this is a reversible processes. In addition, there is a maximum level of adsorption which depends on the available adsorption sites (i.e. media characteristics) and the contact time between the aqueous solution and the filter media. Once the microorganisms are adsorbed to the media during inflow events, they experience die-off during dry weather periods due to a number of factors: desiccation of media, temperature, predation and competition. Because the adsorption is reversible, viable adsorbed microorganisms can be desorbed during subsequent events, and the rate of desorption is thought to be most dependent on the influent characteristics (e.g. ionic strength, pH, flow rate, etc.).

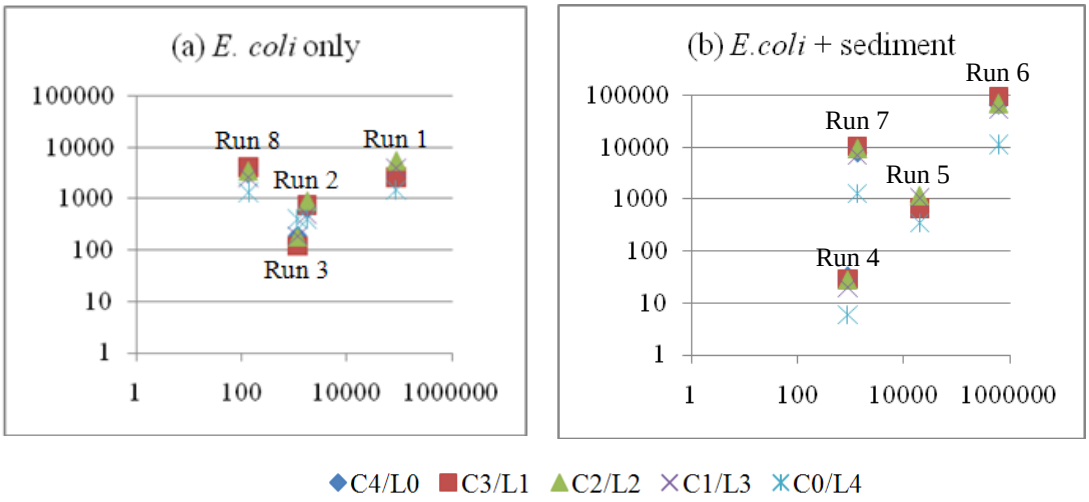

Figure 3. Variation of outflow concentration with inflow concentration in (a) *E. coli* only application and (b) *E. coli* + sediment application. Plant ratio is given as number of *Carex appressa / Lomandra longifolia.*

The above explanation can be further verified by the data shown in Figure 2 and Figure 3. Firstly, a preliminary sampling run conducted using the biofilters showed that negligible *E. coli* were contained with the media before the first sampling run (i.e. 2nd March 2011). Therefore, the difference between the inflow and outflow concentrations of *E. coli* in the first event (88640 MPN/100mL) is hypothesised to be mainly attributed to adsorption processes (i.e. desorption was negligible). In fact, it was estimated that around 1.05×10^7MPN *E. coli* were retained within the filter media during this first event (using the known inflow and outflow concentrations and volumes).

Event 2 occurred only after two days of Event 1, meaning that it is likely that a high number of the adsorbed *E. coli* would be still remaining in the media (this is even if a conservative die-off rate was assumed, e.g. k = 0.16-0.32day^{-1};Bitton and Gerba, 1984). As such, because of desorption/elution effects, it is expected that even if the columns were dosed with '*E. coli* free' water, the level of E. coli in the effluent would be still be significant. In reality, this second event had a low inflow concentration (1791 MPN/100mL) and it is hypothesised that the effluent was now not only a function of the adsorption of these *E. coli* inflowing into the media, but also a function of the desorption of *E. coli* which were remaining in the media from the previous event. Furthermore, the contribution from this background *E. coli* would have been less significant if the current inflow concentration had been higher.

Event 3 occurred five days after the first event and may still have the elution/desorption effect; however, the contribution is expected to be lower than in the second event because some of the *E. coli* has already been desorbed during the second event and there has now been enough time for significant die-off to occur (i.e. 5 days). In fact, the results demonstrate this; while the inflow concentrations for both Event 2 (04/03) and Event 3 (07/03) were similar, the effluent concentrations in Event 3 were lower (Figure 2). This trend continues for Event 4 also (15/03), which showed again a very similar inflow concentration yet significantly lower outflow concentrations than either Event 2 or 3. This is because of the large number of days (9) since the first high concentration inflow event, providing enough time for the majority of the remaining *E. coli* to die-off.

Event 6 (04/04) had very high *E. coli* concentrations and occurred 11 days since the last inflow event. As such, the outflow concentrations during this event are expected to mainly be a function of the adsorption capacity of the media, and the elution/desorption of existing *E. coli* is not going to be a significant contributor since there would have been negligible *E. coli* in the media prior to the event because of the extended dry weather period promoting die-off. In total, it was estimated that around 6.90×10^7MPN were retained within the media during this event, which is considerably more than in Event 1; this finding is consistent with literature, in that adsorption capacity was shown to increase with increasing inflow concentrations (Stevik *et al.*, 2004).

The two subsequent events after Event 6 again reinforced the above discussion. Although Event 7 (06/04) had a similar influent concentration as Events 2, 3 and 4, Event 7's outflow concentrations were around 10 times higher than in these events; this is because of the desorption/elution of the high levels of *E. coli* which remained in the media from Event 6. Event 8 (08/04) had significantly lower inflow concentrations than Event 7, yet Event 8's outflow concentrations were only marginally lower than that of Event 7; this again is because of the elution/desorption of the remaining *E. coli*, but to a lesser extent because of the longer time period available for die-off processes.

The above discussions and understandings are currently being used to develop a predictive model which expands on those currently available (e.g. Zhang *et al.*, 2010). The model focuses on representing the adsorption of *E. coli*, the die-off of these adsorbed *E. coli* during dry weather periods and the subsequent desorption/elution of viable cells from the media during the next event. Current results are promising and confirm that the above hypotheses are correct. However, more data is needed to robustly test and validate the model.

Effect of plant species on removal performance

Table 3 indicates that the treatment of E. coli is impacted by the types of plants present in the biofilter. Generally, *Lomandra longifolia* performs better than *Carex appressa* in all situations, while different mixtures of the two plants show a mixed response but always lower than the performance of *Lomandra longifolia.* This is a relevant finding for the design of these systems for treating other pollutants since *Carex appressa* has a good nutrient removal capacity where as *Lomandra longifolia* is found to have a poorer nutrient removal (Bratieres et al., 2008a, Read et al., 2008). It indicates that the plant traits which promote pathogen removal and nutrient removal may be different. However, further investigation using a range of plants of different traits is warranted and is currently being conducted by the authors.

Table 3. Average log reductions over the dosing period for the different plant configurations. The bolded values indicate the highest average log reduction of the five plant configurations.

Date	Inflow	Plant ratio				
	(MPN/100mL)	C4/L0	C3/L1	C2/L2	C1/L3	C0/L4
02/03/2011	8.66×10^{4}	1.47 (±0.29)	1.54 (±0.14)	1.19 (±0.07)	1.36 (±0.29)	**1.87** (±0.40)
04/03/2011	1.79×10^{3}	0.51 (±0.34)	0.44 (±0.38)	0.31 (±0.22)	0.59 (±0.26)	**0.75**(±0.41)
07/03/2011	1.17×10^{3}	0.84 (±0.29)	1.00 (±0.16)	0.88 (±0.46)	0.83 (±0.35)	**1.24** (±0.42)
15/03/2011	8.84×10^{2}	1.72 (±0.74)	1.75 (±0.61)	1.56 (±0.47)	1.68 (±0.32)	**2.19** (±0.217)
23/03/2011	2.01×10^{4}	1.47 (±0.46)	1.73 (±1.16)	1.25 (±0.22)	1.38 (±0.63)	**2.13** (±0.81)
04/04/2011	6.25×10^{5}	1.17 (±0.63)	1.36 (±1.82)	1.19 (±0.89)	1.13 (±0.51)	**1.93** (±0.56)
06/04/2011	1.36×10^{3}	-0.70 (±0.33)	-0.38 (±1.54)	-0.82 (±0.27)	-0.63 (±0.54)	**0.09** (±0.40)
08/04/2011	1.36×10^{2}	-1.29 (±0.35)	-0.99 (±2.07)	-1.37 (±0.33)	-1.27 (±0.21)	**-0.93** (±0.46)

Average log reduction (95% confidence interval)

CONCLUSIONS

It can be concluded that the removal performance of stormwater biofilters are highly affected by the inflow concentrations and antecedent concentrations. However, this should be further investigated and more data should be collected to confirm the hypotheses made within this paper. Furthermore, future tests should also study the removal of microorganisms using natural stormwater and actual pathogenic organisms. The selection of plant species was found to be important in *E. coli* removal. Future research focused on improving biofiltration systems should not only consider mechanisms to enhance retention but also into mechanisms which promote rapid inactivation of captured microorganisms. Certain plants, which release antimicrobial agents, should be investigated to determine whether they can enhance this inactivation during dry weather periods.

ACKNOWLEDGEMENT

The authors wish to acknowledge the support of Tracey Pham, Javier Neira and Centre for Water Sensitive Cities, Monash Sustainability Institute, Monash University.

REFERENCES

BITTON, G. & GERBA, C. P. 1984. Microbial Pollutants: Their Survival and Transport Pattern to Groundwater. *In:* BITTON, G. & GERBA, C. P. (eds.) *Groundwater Pollution Microbiology.* New York: John Wiley and Sons.

BRATIERES, K., FLETCHER, T. D., DELETIC, A., ALCAZAR, L., COUSTUMER, S. L. & MCCARTHY, D. T. 2008a. Removal of nutrients, heavy metals and pathogens by stormwater biofilters. *11th International Conference on Urban Drainage.* Edinburgh, Scotland, UK.

BRATIERES, K., FLETCHER, T. D., DELETIC, A. & ZINGER, Y. 2008b. Nutrient and sediment removal by stormwater biofilters: A large-scale design optimisation study. *Water Research,* 42**,** 3930-3940.

BRIX, H. 1997. Do macrophytes play a role in constructed treatment wetlands?

CHARACKLIS, G. W., DILTS, M. J., SIMMONS III, O. D., LIKIRDOPULOS, C. A., KROMETIS, L.-A. H. & SOBSEY, M. D. 2005. Microbial partitioning to settleable particles in stormwater. *Water Research,* 39**,** 1773-1782.

DAVIES, C. M. & BAVOR, H. J. 2000. The fate of stormwater-associated bacteria in constructed wetland and water pollution control pond systems. *Journal of Applied Microbiology,* 89**,** 349-360.

FAWB 2009. Adoption Guidelines for Stormwater Biofiltration Systems. Facility for Advancing Water Biofiltration, Monash University.

FERGUSON, C., DE RODA HUSMAN, A. M., ALTAVILLA, N., DEERE, D. & ASHBOLT, N. 2003. Fate and transport of surface water pathogens in watersheds. *Critical Reviews in Environmental Science and Technology,* 33**,** 299-361.

HATHAWAY, J. M., HUNT, W. F., WRIGHT, J. D. & JADLOCKI, S. J. Year. Field evaluation of indicator bacteria removal by stormwater BMPs in North Carolina. *In*, 2009 Kansas City, MO. 1123-1132.

HATT, B. E., FLETCHER, T. D. & DELETIC, A. 2009. Pollutant removal performance of field-scale stormwater biofiltration systems. *Water science and technology* 59**,** 1567-1576.

IDEXX-LABORATORIES 2007. Colilert® Test Kit. Maine, USA: IDEXX-Laboratories.

ISHII, S., KSOLL, W. B., HICKS, R. E. & SADOWSKY, M. J. 2006. Presence and growth of naturalized Escherichia coli in temperate soils from lake superior watersheds. *Applied and Environmental Microbiology,* 72**,** 612-621.

READ, J., WEVILL, T., FLETCHER, T. & DELETIC, A. 2008. Variation among plant species in pollutant removal from stormwater in biofiltration systems. *Water Research,* 42**,** 893-902.

RUSCIANO, G. M. & OBROPTA, C. C. 2007. Bioretention column study: Fecal coliform and total suspended solids reductions. *Transactions of the ASABE,* 50**,** 1261-1269.

SCHILLINGER, J. E. & GANNON, J. J. 1985. Bacterial adsorption and suspended particles in urban stormwater. *Journal of the Water Pollution Control Federation,* 57**,** 384-389.

STEVIK, T. K., AA, K., AUSLAND, G. & HANSSEN, J. F. 2004. Retention and removal of pathogenic bacteria in wastewater percolating through porous media: A review. *Water Research,* 38**,** 1355-1367.

STRUCK, S. D., SELVAKUMAR, A. & BROST , A. 2006. Performance of Storm Water Retention Ponds and Constructed Wetlands in Reducing Microbial Concentrations. Washington DC: U. S. Environmental Protection Agency.

WONG, T. H. F. 2006. *Australian runoff quality : a guide to water sensitive urban design* Engineers Australia.

ZHANG, L., SEAGREN, E. A., DAVIS, A. P. & KARNS, J. S. 2010. The capture and destruction of Escherichia coli from simulated urban runoff using conventional bioretention media and iron oxide-coated sand. *Water environment research : a research publication of the Water Environment Federation,* 82**,** 701-714.