Framework for real time control and operational optimisation of stormwater biofilters

Pengfei Shen

A thesis submitted for the degree of
Doctor of Philosophy

Department of Civil Engineering
Monash University

March 2019

Copyright notice

I, Pengfei Shen, certify that I have made all reasonable efforts to secure copyright permissions for third-party content included in this thesis and have not knowingly added copyright content to my work without the owner's permission.

Abstract

Stormwater is an alternative water resource for local communities. However, it needs to be treated before harvesting and reuse, as the faecal microbes contained in stormwater could pose considerable risks to human health. Stormwater biofilters are widely applied as a water sensitive urban design system for stormwater treatment, but their performance is inconsistent, although much effort has been placed in optimising their design features. The operational conditions in biofilters (e.g., retention time and inflow volume) are also crucial and are a major driver of the net export of faecal organisms which is often observed. To achieve operational optimisation, Real time control (RTC) is a potential technique, and this study aims to develop a framework for RTC and the operational optimisation of biofilters.

A process-based model was first developed to simulate long-term microbial removal in stormwater biofilters. The model employs one-dimensional advection-dispersion equations to represent microbial transport and fate under different design and operational conditions. By testing the model with the laboratory data collected from five biofilter configurations, the prediction showed good agreement with observation.

In the second part of this study, the developed model was validated with the results of extensive laboratory experiments and field tests. Although the lab-scale and field-scale systems used for validation have various designs and operational conditions, the model could consistently provide good prediction of faecal microbial removal. More importantly, this model is highly transferable, providing accurate prediction with generally low uncertainty when calibrated with one dataset (e.g., a laboratory dataset) and applied to another independent dataset (e.g., a field dataset).

In the third part, this study provides the first evidence that RTC of stormwater biofilters can regulate the operational characteristics that result in poor microbial removal. Two RTC strategies were developed and tested via

laboratory experiments, and they were both found to be effective in reducing the risks posed by faecal microbes. However, competing needs were found in stormwater harvesting and reuse, including the harvested water volume, harvested water quality, and environmental protection.

As such, additional RTC strategies and scenarios were explored in the last part of this study to balance the competing needs. In this part, a new model, BioRTC, was developed for RTC simulation by modifying the model introduced in the first part of this study. It was found that BioRTC could reflect the outcome of RTC implementation well when being calibrated with the data collected in the third part of this study. With the help of this modelling tool, two additional RTC strategies were conceptualised and assessed in modelling scenarios, and both were found to be effective in balancing the competing needs in stormwater harvesting and reuse.

This study presents the first stepping stone toward RTC of stormwater biofilters, demonstrating that RTC can help to deliver safe water for harvesting and reuse, and for active recreational uses. Moreover, an efficient modelling framework tool, BioRTC, was developed for stormwater biofiltration RTC strategy evaluation and selection, enabling a broad exploration in this new field.

Thesis including published works declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes one original paper published in a peer-reviewed journal (Chapter 3) and one submitted publication (Chapter 4). The core theme of the thesis is the application of real time control in stormwater biofilters to achieve operational optimisation, in order to maximise biofilters' function in stormwater harvesting and reuse. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the student, working within the Department of Civil Engineering under the supervision of Associate Professor David McCarthy, Professor Ana Deletic, and Dr Christian Urich.

For Chapter 4, the contribution of the co-authors can be described as follows: David McCarthy: research formulation and editing; Ana Deletic: research formulation and editing; Gayani Chandrasena: data analysis; Yali Li: data analysis. For Chapter 5, the contribution of the co-authors is: David McCarthy: research formulation and editing; Ana Deletic: research formulation. The contribution of co-authors for Chapter 6 consisted in the following: David McCarthy: research formulation and editing; Ana Deletic: research formulation.

The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.

In the case of Chapter 3, my contribution to the work involved the following:

Thesis Chapter	Publication Title	Status	Nature and % of student contribution	Co-author name(s) Nature and % of Co-author's contribution*	Co-author(s), Monash student Y/N*
3	Stormwater biofilter treatment model for faecal microorganisms	Published	60 %: research formulation, data collection, modelling, writing	1) David McCarthy: research formulation, model development, editing (17.5 %) 2) Ana Deletic: editing (7.5 %) 3) Christian Urich: modelling (7.5 %) 4) Gayani Chandrasena: data analysis (7.5 %)	No

I have/have not renumbered sections of submitted or published papers in order to generate a consistent presentation within the thesis.

Student signature: [redacted] **Date:** 20th November, 2018

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the student's and co-authors' contributions to this work. In instances where I am not the responsible author, I have consulted with the responsible author to agree on the respective contributions of the authors.

Main Supervisor signature: [redacted] **Date:** 22nd November, 2018

Acknowledgements

I would first like to express my sincere gratitude to my supervisors, David McCarthy, Ana Deletic, and Christian Urich. All of them have provided me with utmost helpful guidance and support throughout my PhD. David is the most intelligent person I have ever worked with. Ana is an outstanding and far-sighted scientist (also with the best cooking skills). Christian has amazing expertise in computer science. I have learnt and gained so much from them, and it is my greatest honour to have an opportunity to work with them.

I would also like to thank all the members from EPHM Lab and the Water group of Civil Engineering, Monash University. Almost everyone in these fantastic groups has assisted me. Big thanks to Edoardo Daly, Belinda Hatt, Peter Bach, Christelle Schang, Rebekah Henry, Gayani Chandrasena, Juri Jung, Harsha Fowdar, Wenjun Feng, Anna Lintern, Veljko Prodanovic, Penelope Galbraith, Yali Li, Richard Williamson, and Tony Brosinsky for all the help during my modelling and laboratory studies. I also want to express the deepest appreciation to Emily Payne, Lynze Cheung, Rob Sargent, and Baiqian Shi for lending me a hand to structure and polish my papers and thesis. It is a great pleasure to work and build the friendship with all of them.

I gratefully acknowledge the Monash Faculty of Engineering and the Department of Civil Engineering for their funding and support. In particular, the funding that supported me to conduct research at Stanford University enabled me to experience a very enjoyable adventure.

I also acknowledge all the GRIP members, who have made this PhD study a smoother and more pleasant endeavour. Special thanks to Adam Castonguay and his wife Wenting Han – I will always remember the delightful days when we were cooking, travelling, playing games, and discussing research together.

Many thanks to Luca Vezzaro from the Technical University of Denmark, who is the panel member for all the milestones of my PhD study and provided me with fantastic suggestions.

I truly appreciate my previous supervisor during my Master's study, Luming Ma, for his continuous concern and support. I consider him as not only my mentor, but also a considerate and gracious family member.

Special thanks to Yan You for her understanding and encouragement. Her concern, console, and witty words lightened the most stressful days of my PhD study, enabling me to overcome the hardship.

I am also grateful to my warm-hearted friends, such as Jingming Qian, Lin Chen, Wei Liu, Yunfeng Mao, Minyu Fu, Wei Gao, and Jiayou Ming, for their concern and help. Thanks to Bing Han - without her support I might not even have a chance to pursue this PhD.

Lastly, I would like to express my deepest gratitude to my parents to their unconditional support. 感谢你们，我亲爱的爸爸妈妈。

Table of contents

Nomenclature

Acronyms / Abbreviations

ANOVA	Analysis of variance
CP	*Carex appressa*
E. coli	*Escherichia coli*
LC	*Leptospermum continentale*
LS	Loamy sand
PB	*Palmetto buffalo*
PV	Pore volume
PZ	Ponding zone
RTC	Real time control
RTM	Real time monitoring
SD	Standard deviation
SZ	Submerged zone / Saturated zone
TN	Total nitrogen
TP	Total phosphorus
TSS	Total suspended solids
USZ	Unsaturated zone
WS	Washed sand
WSUD	Water sensitive urban design

Chapter 1.

Introduction

1.1 Introduction

Stormwater runoff may cause flooding, degradation of urban streams, and public health risks (Wong, 2006). However, it could serve as an alternative water resource, especially considering that more than half of the high-quality water supply is used only for lower quality purposes (Wood et al., 2002). Importantly, the amount of stormwater runoff from Australian cities is about equal to the amount of drinking water use (Wood et al., 2002). However, the wide range of pollutants contained in stormwater impede its harvesting and reuse (Fletcher et al., 2008). In particular, faecal microbes are a major concern, as they pose health risks when contacting with human bodies (NHMRC, 2009). To control the risks caused by faecal microbes, stormwater needs to be treated before harvesting and reuse.

To treat faecal microbes and other pollutants in stormwater runoff, water sensitive urban design (WSUD) systems have been widely employed. Among them, stormwater biofilters are especially promising and have been commonly adopted (FAWB, 2009). Stormwater biofilters are soil-plant based systems with enhanced infiltration and evapotranspiration (FAWB, 2009). The major processes for microbial removal in stormwater biofilters include adsorption, desorption, straining, and die-off (Stevik et al., 2004). These processes are also governed by various design and operational factors, such as plant type, media type, temperature, and moisture content (Chandrasena et al., 2014a; Ferguson et al., 2003; Stevik et al., 2004). However, the microbial removal rate of stormwater biofilters under different conditions is inconsistent (Chandrasena et al., 2014b; Li et al., 2012; Zhang et al., 2010; Zhang et al., 2011).

To enhance the microbial removal in stormwater biofilters, much effort has been placed in optimising the design of biofilters, such as testing the influences of different plants, various filter media, and the existence of submerged zone (Chandrasena et al., 2014b; Li et al., 2016; Li et al., 2012). Unfortunately, the optimisation of design could not mitigate impact of some extreme weather conditions, such as the events with a large amount of inflow volume, long antecedent dry period, or short antecedent dry period.

Importantly, very little has been done to control the operational conditions that are experienced by biofilters (e.g., infiltration rate and retention time), even though they are found to govern the microbial removal in stormwater biofilters (Chandrasena et al., 2014a). Moreover, operational optimisation would be a solution to enable biofilters being adjustable to various weather conditions. However, the operational conditions of biofilters, unlike the design, are difficult to control, as stormwater biofilters have been traditionally designed as passive treatment systems.

Real time control (RTC) is a potential technology to make stormwater biofilters "active" for the optimisation of their performance by controlling operational conditions. The general concept of RTC is monitoring of the functioning of a system in real time and using this data to optimise performance through the control of the certain aspects of the system (Schütze et al., 2004). RTC has been widely used and proved to be effective in other studies in environmental field, such as flooding control, capacity enlargement of sewer systems, and wastewater treatment optimisation (Hsu et al., 2015; Leon et al., 2014; Schilling et al., 1996; Schütze et al., 2004). However, no publication have been found on the study of applying RTC in stormwater biofilters. Therefore, it is necessary to develop a framework for RTC of stormwater biofilters.

1.2 Overall aim and tasks

The overall aim of this study is to develop a framework for RTC and operational optimisation of stormwater biofilters, in order to provide better outflow quality of biofilters and reduce the risks posed by faecal microbes in stormwater harvesting and reuse. To achieve the aim of this study, three tasks are specified and need to be fulfilled:

(1) Develop and validate a process-based model for the prediction of microbial removal in stormwater biofilters;

(2) Develop RTC strategies to optimise microbial removal in biofilters for stormwater harvesting and reuse, and test the developed strategies with laboratory column studies;

(3) Create a new model for RTC by modifying the developed model, validate the new model using the data collected from RTC experiments, and explore additional RTC strategies and scenarios.

1.3 Scope of the research

In this study, the major purpose of adopting RTC is to optimise the removal of microbial concentration in stormwater biofilters' outflow, as faecal microbes are considered as one of the most risky pollutants in stormwater harvesting and reuse. Although other pollutants that contained in stormwater runoff, such as suspended solids, nitrogen and phosphorous, have also been tested during some events in laboratory experiments, the removal effects on these pollutants are not the primary criteria in RTC evaluation and selection in this study.

Various types of faecal microbes have been detected in stormwater runoff; however, *Escherichia coli* (*E. coli*), is selected as the indicator of faecal microbes in this study. The reasons include: (1) *E. coli* is one of the most widely used indicators and has been selected as a target pollutant in various water quality guidelines; and (2) the governing processes for *E. coli* removal (e.g., adsorption, desorption, straining and die-off) are also applicable to other types of microbes (although the importance of each individual process may vary for different types of microbes), therefore, the optimisation of *E. coli* removal is assumed to be effective for the removal other microbes.

The study integrates both modelling and experiments. For the experiments part, only laboratory-scale column studies are conducted, as compared to field tests, (a) the operational conditions in the laboratory are much easier to control, (b) the implementation of RTC in the laboratory is considerably more repeatable, and (c) the monitoring uncertainty in the laboratory is much lower.

The implementation of one of the RTC strategies developed in this study (named as Harvesting-Environment RTC) incorporates rainfall forecast information; however, since the historical data of rainfall prediction are not available, this study only focuses on the "pure" benefits of RTC by assuming

that only perfect rainfall forecast information is obtained; the extra uncertainties caused by inaccurate rainfall forecasting are out of the scope of this study, but are expected to be investigated in the future work.

1.4 Outline of the thesis

The thesis will consist of seven chapters. An overview of each chapter is provided below.

Chapter 1: Introduction

This chapter introduces the background and purpose of this study, overall research aim and objectives, scope of the research, and culminates in providing the outline of the thesis.

Chapter 2: Literature review

A detailed review of literature on the research topic will form the content of this chapter. The major structure of this chapter is:

1. Introduction
2. Microorganisms in stormwater runoff
3. Water sensitive urban design systems for stormwater treatment
4. Governing processes and factors for microbial removal in stormwater biofilters
5. Real time control of stormwater biofilters
6. Predictive models for microbial removal in stormwater biofilters
7. Conclusions of literature review and key knowledge gaps
8. Research questions and hypotheses
9. References

Chapter 3: Development and testing of stormwater biofilter treatment model for faecal microorganisms

This chapter presents the development of a model for the prediction of microbial removal in stormwater biofilters. The equations and parameters of this model are described in detail. This chapter also explained the model testing procedure, including how the experimental data that used for calibration are

collected, as well as the calibration, parameter selection, and sensitivity analysis processes. This chapter also reports the results of model validation attempts when using the same data sets for model development.

Chapter 4: Model validation, parameter transferability analysis, and uncertainty analysis

Since in Chapter 3, the model has not been successfully validated due to data paucity and more data are suggested to be included in sensitivity analysis and model validation, this chapter presents the process of a more comprehensive validation of the model that developed in Chapter 3. In this chapter, additional two data sets, one is collected from laboratory experiments and the other is collected from field testing, are utilised to validate the model. Calibration and sensitivity analysis results are presented in this chapter. In addition, this chapter includes parameter transferability and prediction uncertainty analyses, to assess the performance of adopting the parameter sets calibrated in one system for the prediction of another system with a similar design.

Chapter 5: Development and laboratory testing of real time control strategies for stormwater biofilters

This chapter introduces the development of two RTC strategies for stormwater biofilters, Harvesting RTC and Harvesting-Environment RTC. The rules and control processes of each strategy are explained in detail. In addition, this chapter reports the set-up, conduction process, and the results of the laboratory column experiments for the testing of these two RTC strategies, to reveal the benefits of each strategy.

Chapter 6: Model modification and validation for real time control, and further exploration of control strategies

The chapter presents the study of modifying the model developed in Chapter 3 for RTC simulation, and validating the modified model with the data collected from the laboratory experiments that presented in Chapter 5, to evaluate the feasibility of employ the model for RTC assessment and selection. In addition, to balance the competing needs in stormwater harvesting and reuse, two additional RTC strategies are conceptualised based on the two strategies

developed and tested in Chapter 5. Moreover, this chapter shows the results of simulating the two newly conceptualised strategies and the two previously developed strategies in the scenarios that have a modelled representation of reality as inputs.

Chapter 7: Conclusions and future work

This chapter summarises the key findings in modelling and experiments. Conclusions and practical implications, and strengths and limitations of this study are highlighted. Recommendations for future work are also provided.

1.5 References

Chandrasena G. I., Deletic A., McCarthy D. T. Survival of *Escherichia coli* in stormwater biofilters. Environmental Science and Pollution Research 2014a; 21: 391-401.

Chandrasena G. I., Pham T., Payne E. G., Deletic A., McCarthy D. T. *E. coli* removal in laboratory scale stormwater biofilters: Influence of vegetation and submerged zone. Journal of Hydrology 2014b; 519: 814-822.

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

Ferguson C., De Roda Husman A. M., Altavilla N., Deere D., Ashbolt N. Fate and transport of surface water pathogens in watersheds. Critical Reviews in Environmental Science and Technology 2003; 33: 299-361.

Fletcher T. D., Deletic A., Mitchell V. G., Hatt B. Reuse of urban runoff in Australia: a review of recent advances and remaining challenges. Journal of Environmental Quality 2008; 37: 116 - 127.

Hsu N.-S., Huang C.-L., Wei C.-C. Multi-phase intelligent decision model for reservoir real-time flood control during typhoons. Journal of Hydrology 2015; 522: 11-34.

Leon A. S., Kanashiro E. A., Valverde R., Sridhar V. Dynamic Framework for Intelligent Control of River Flooding: Case Study. Journal of Water Resources Planning and Management 2014; 140: 258-268.

Li Y., McCarthy D. T., Deletic A. *Escherichia coli* removal in copper-zeolite-integrated stormwater biofilters: Effect of vegetation, operational time, intermittent drying weather. Ecological Engineering 2016; 90: 234-243.

Li Y. L., Deletic A., Alcazar L., Bratieres K., Fletcher T. D., McCarthy D. T. Removal of Clostridium perfringens, Escherichia coli and F-RNA coliphages by stormwater biofilters. Ecological Engineering 2012; 49: 137-145.

NHMRC. Australian Guidelines for Water Recycling (Phase 2). Stormwater Harvesting and Reuse. Australian Government, Natural Resource Management Ministerial Council, Environment Protection and Heritage Council, National Health and Medical Research Council, Canberra, Australia, 2009.

Schilling W., Andersson B., Nyberg U., Aspegren H., Rauch W., Harremoës P. Real time control of wastewater systems. Journal of Hydraulic Research 1996; 34: 785-797.

Schütze M., Campisano A., Colas H., Schilling W., Vanrolleghem P. A. Real time control of urban wastewater systems—where do we stand today? Journal of Hydrology 2004; 299: 335-348.

Stevik T. K., Aa K., Ausland G., Hanssen J. F. Retention and removal of pathogenic bacteria in wastewater percolating through porous media: a review. Water Research 2004; 38: 1355-1367.

Wong T. H. F., Australia E. Australian runoff quality: a guide to water sensitive urban design. Crows Nest, N.S.W., Australia: Engineers Media for Australian Runoff Quality Authorship Team, 2006.

Wood J., Kelly T., Moran M. Introduction to Urban Stormwater Management in Australia. Department of the Environment and Heritage, Australia, 2002.

Zhang L., Seagren E. A., Davis A. P., Karns J. S. The Capture and Destruction of *Escherichia coli* from Simulated Urban Runoff Using Conventional Bioretention Media and Iron Oxide-coated Sand. Water Environment Research 2010; 82: 701-714.

Zhang L., Seagren E. A., Davis A. P., Karns J. S. Long-term sustainability of *Escherichia coli* removal in conventional bioretention media. Journal of Environmental Engineering 2011; 137: 669-677.

Chapter 2.

Literature review

2.1 Introduction

This chapter starts with introducing different types of microorganisms in stormwater runoff and the indicators of them, with a connection to the corresponding treatment requirements in guidelines in Australia. Afterwards, the design and performance of different water sensitive urban design (WSUD) systems in stormwater treatment are analysed, with a special focus on stormwater biofilters. These are followed by the description of governing processes and factors for microbial removal in stormwater biofilters. The next section of this chapter introduces the concept of real time control (RTC), and the benefits and lessons of implementing RTC in other areas of environmental field, with a summary of the control categorisation. Based these discussions, a framework for the application of RTC in stormwater biofilters is proposed, with the key points in implementation being outlined. Since predictive models serve as the fundamental part of RTC, current models for microbial removal in stormwater biofilters are also discussed, highlighted the necessity of developing a microbial removal model to achieve RTC in stormwater biofilters. After summarising the conclusions of literature review and key knowledge gaps, this chapter culminates with research questions and hypotheses.

2.2 Microorganisms in stormwater runoff

Numerous types of contaminants have been detected in urban stormwater runoff, including nitrogen, phosphorous, heavy metals, suspended solids, micropollutants, and microorganisms (also named as microbes) (Duncan, 1999; Makepeace et al., 1995). Among them, microorganisms are especially risky to human health (NHMRC, 2004). In particular, faecal microorganisms, which are sourced from the faeces of animals and humans, are considered as the cause of waterborne diseases and present a higher degree of risk to end uses compared to non-faecal microorganisms (i.e., the microorganisms are not from the intestines of animals) (Meng et al., 2018; Murphy et al., 2017). As such, in this study, the target pollutant is faecal microorganisms.

There are three main types of microorganisms: bacteria, protozoa and viruses. Bacteria are single-celled organisms with a number of shapes, which can exist

either as a free phase or associated with other particles; protozoa are also single-celled but generally have a larger size (2 ~ 100 μm) compared to bacteria; viruses can only reproduce within their host and have the smallest size (0.02 ~ 0.09 μm) among the three types (Perdek et al., 2003). Table 2.1 lists the typical microorganisms in stormwater with their negative effects to health and reported concentrations.

Table 2.1 Typical microorganisms in stormwater with their negative effects to health and reported concentrations (adapted from Chandrasena (2014)).

	Microorganism	Negative effect to health	Reported concentration in stormwater (cells/L)
Bacteria	*Campylobacter*	Campylobacteriosis (1)	$10 \sim 7.0 \times 10^{2}$ (4)
	Escherichia coli (OH157:H7)	Gastroenteritis (1)	$1.2 \times 10^{2} \sim 1.3 \times 10^{6}$ (2)
	Klebsiella	Infections and pneumonia(2)	$4.0 \times 10^{4} \sim 1.9 \times 10^{6}$ (3)
	Psedomonas aeruginosa	Eye irritations (2), ear and skin rashes (3)	$10 \sim 1.2 \times 10^{4}$ (3,5)
	Salmonella	Salmonellosis (1)	$1.7 \times 10^{-1} \sim 4.5 \times 10^{5}$ (3,6)
	Staphylococcus aureus	Skin rashes, boils, impetigo (3)	$10 \sim 1.8\times10^{5}$ (3,5)
Protozoa	*Cryptosporidium*	Cryptosporiduosis (1)	$8.0 \times 10^{-2} \sim 2.9 \times 10^{-1}$ (7)
	Giardia	Giardiasis (1)	$8.0 \times 10^{-2} \sim 3.0 \times 10^{-1}$ (7)
Virus	Adenovirus	Respiratory disease, gastroenteritis (1)	$< 1.0 \times 10^{-1}$ (4)
	Coxsackie virus B	Gastroenteritis (1)	18 (3)
	Enterovirus	Gastroenteritis, heart anomalies, meningitis (1)	$6.9 \times 10^{-1} \sim 28$ (3,6)
	Reovirus	Gastroenteritis (1)	61 (3)

Sources: (1)Arnone and Walling (2007); (2)NHMRC (2004); (3)Makepeace et al. (1995); (4)NHMRC (2009); (5)WERF (2007); (6)Oliveri et al. (1977); (7) Ecowise (2010).

2.2.1 Indicator microorganisms

Considering the large number of microorganism types (Duncan, 1999; Makepeace et al., 1995), the relatively low concentration of each

microorganism type (Duncan, 1999; Makepeace et al., 1995), and the high cost for measurement (Brookes et al., 2005; Horan, 2003), detecting all the types of microbes in stormwater is unpractical. Therefore, indicators for microorganisms are normally used to indicate the level of faecal contamination in water (Brookes et al., 2005; Horan, 2003). An ideal indicator should: (1) present in water when microorganisms are present, (2) present with a higher number compared to microorganisms, (3) have similar persistence to environmental conditions with microorganisms, and (4) multiply in environment (Cizek et al., 2008; Horan, 2003; Schijven and Hassanizadeh, 2000).

It is noted that, no indicator microorganism could have all these properties and be considered as “ideal”, and each indicator microorganism may have both advantages and disadvantages. Therefore, different indicator microorganisms were selected in different studies. The most widely used ones include the coliform group, such as *Escherichia coli*, enterococci, *Bacteroides spp.*, *Bifidobacterium spp.*, *Clostridium perfringens* and *Bacteriophages*. (Brookes et al., 2005; Horan, 2003; NHMRC, 2004; Schijven and Hassanizadeh, 2000). Especially, *E. coli* (*Escherichia coli*) that from the coliform group, is widely selected as an indicator for faecal microorganism due to several advantageous characteristics: exclusively associated with a faecal source, inability to multiply in different environments, and easy and cheap to be detected with enzymatic methods (Edberg et al., 2000). Table 2.2 lists some common indicator microorganisms and their concentrations in stormwater.

Table 2.2 Types and concentrations of indicator microorganisms reported in stormwater (adopted from Chandrasena (2014)).

	Indicator	Reported concentration in stormwater (cells/L)
Bacteria	Total coliforms	$7.0 \times 10^{-1} \sim 1.8 \times 10^{8}$ (1)
	Faecal coliforms	$2.0 \sim 3.0 \times 10^{7}$ (1, 2)
	E. coli	$1.0 \sim 1.4 \times 10^{6}$ (3)
	Faecal Streptococci	$30 \sim 3.8\times10^{7}$ (1,4)
	enterococci	$1.0 \times 10^{3} \sim 3.4 \times 10^{6}$ (1, 2)
	Clostridium perfringens	$1.7 \times 10^{-1} \sim 4.5 \times 10^{5}$ (3, 6)
Virus	Somatic coliphage	$10 \sim 5.5 \times 10^{5}$ (4,5)
	FRNA coliphage	$10 \sim 2.1 \times 10^{3}$ (4)

Sources: (1) Makepeace et al. (1995); (2) WERF (2007); (3) Ecowise (2010); (4) Oliveri et al. (1977); (5) NHMRC (2009);(6) Davies et al. (2003).

2.2.2 Treatment Requirements in Australia

To reduce the risks of pathogens posed on humans, Australia established several guidelines for microorganisms control in stormwater harvesting, drinking water and recreational water bodies (Table 2.3). These guidelines all base on indicator microorganisms, and *E. coli* is the most commonly used indicator for stormwater harvesting.

Table 2.3 Requirements for microbial concentrations in water in Australian guidelines (adopted from Chandrasena (2014)).

Guideline	End use	Requirement
Australian guidelines for stormwater harvesting and reuse (NHMRC, 2009)	Municipal use, with unrestricted access (open spaces, sports grounds, golf courses, and non-potable construction uses) or irrigation of non-food crops	< 10 *E. coli*/100 mL
	Dual reticulation with indoor or outdoor use or irrigation of commercial food crops	< 1 *E. coli*/100 mL
Australian Drinking Water Guidelines (NHMRC, 2004)	Drinking water (potable water)	no *E. coli* detected
Australian Guidelines for Managing Risks in Recreational Water (NHMRC, 2008)	Primary contact (swimming, bathing and other direct water-contact sports)	< 150 faecal coliform/100 mL < 35 enterococci/100 mL 0 Pathogenic free-living protozoan
	Secondary contact (boating and fishing)	< 1000 faecal coliform/100 mL < 230 enterococci/100 m

By comparing the microbial concentrations report in stormwater with the treatment requirements in guidelines, it can be concluded that the treatment of stormwater for microbial removal is essential.

2.3 Water sensitive urban design systems for stormwater treatment

To fulfil the requirements in aforementioned guidelines that listed in Section 2.2.2, several types of water sensitive urban design (WSUD) systems have been developed. WSUD is a concept that integrates holistic management of urban water cycle (i.e., potable water, wastewater, and stormwater) into design, and one objective is to treat urban stormwater to meet the water quality requirements for harvesting and waterbody protection (Wong, 2006).

There are various types of end uses of WSUD systems (Figure 2.1) (Hatt et al., 2006). In Figure 2.1, “other outdoor use” includes domestic use like car washing and window washing, whilst “other use” includes industrial reuse, commercial bus washing, and groundwater recharge. Figure 2.1 shows that, irrigation of public spaces and gardens is the most common type of end use (44 %), and 72 % stormwater is used for irrigation, toilet flushing, and other outdoor uses.

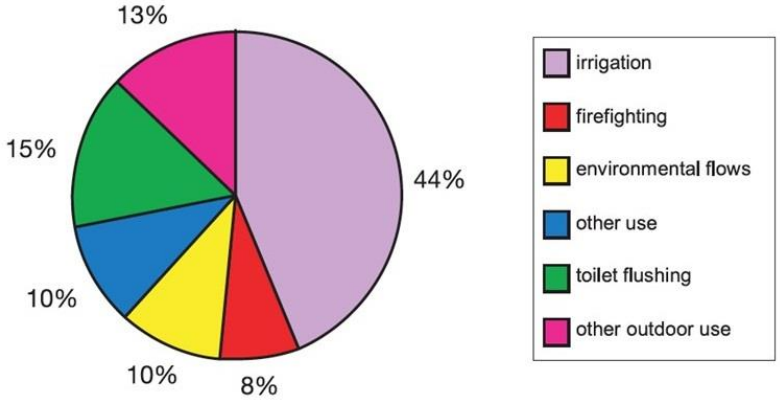


Figure 2.1 Stormwater end use types and the volume percentage of each use (adopted from Hatt et al. (2006)).

2.3.1 WSUD systems in faecal microbial removal

To meet the requirements for the end uses listed in Figure 2.1, different types of WSUD systems have been adopted, including stormwater biofilters, wetlands, vegetated swales, and stormwater retention ponds.

Stormwater biofilters, also known as biorentention systems or rain gardens, are one of the most widely used WSUD systems in Australia and other countries for stormwater treatment (FAWB, 2009). Stormwater biofilters are soil-plant, natural based systems with the enhancement of infiltration and evapotranspiration of stormwater. Therefore, they are low-energy treatment systems with the potential to provide both water quantity and quality benefits (FAWB, 2009). The treatment of stormwater in biofilters is a combination of physical, (e.g. mechanical straining), chemical (e.g. adsorption) and biological (e.g. plant uptake) processes (FAWB, 2009). Biofilters have been proved to be effective in the removal of various pollutants: e.g., 57 ~ 93 % for total suspended solids (TSS), 30 ~ 55 % total nitrogen (TN) and 5 ~ 80 % for total phosphorus (TP) (CWSC, 2010). For microbial removal, stormwater biofilters are often

effective in laboratory studies, as the removal rate for *E. coli* ranged from 73% to 99.9% (Chandrasena et al., 2014b; Li et al., 2012; Zhang et al., 2010; Zhang et al., 2011). However, in field studies, the *E. coli* removal rate was found to be far more variable - it could range from 99% to even leaching (< 0) under challenging operational conditions (Chandrasena et al., 2016; Hathaway et al., 2011; Hathaway et al., 2009; Zinger et al., 2011). Further examples existed for other reference pathogens such as *Campylobacter* spp.: e.g., Chandrasena et al. (2016) reported its removal rate ranged from -90.5 % to 99.1 %.

Wetlands are also utilised for stormwater treatment as a WUSD system. It is operated either as standalone facilities or in the combination with other WSUD systems (e.g., stormwater retention ponds). Wetlands have been reported to be effective in promoting oxygen recovery, and trapping sediment, nutrients, bacteria, and toxins (Malaviya and Singh, 2012). The major elements of a wetland include pre-treatment, inlet pond, ephemeral zone, and wetland zone; stormwater could be treated in these elements with a combination of sedimentation, filtration, and biological nutrient uptake. The key factors for treatment effects include wetland shape, storage volume/residence time, soil type, species type and number of aquatic plants (Kadlec and Wallace, 2008). Studies suggested that the removal rate of TSS varies between 58 ~ 85%; the removal of TN is 16 ~ 30% and the removal of TP is 46 ~ 60% (Carleton et al., 2000; Wadzuk et al., 2010). In addition, the microbial removal efficiency of wetlands reported in previous studies varied significantly: take *E. coli* removal for example, Díaz et al. (2010) and Stenstrom and Carlander (2001) respectively indicated a removal efficiency of 80 ~ 95% and 99%. However, some other studies even found an increase of *E. coli* concentration after treatment (Hathaway and Hunt, 2010).

Vegetated swales are open, grassed surfaces that aim to reduce runoff velocity and retain coarse sediments (Hatt et al., 2004). Vegetated swales collect and treat stormwater by filtration; they can also serve as a pre-treatment process for other WSUD technologies. They are effective in removal of suspended solids, but the performance in nutrients and heavy metals removal is lower and unstable (Fletcher et al., 2007). In particular, some studies reported that a

consistent increase of pathogen concentrations was observed, even when the access of faecal bacteria to the swales was limited (CWSC, 2010).

Stormwater retention ponds are designed for stormwater runoff interception before runoff reaches water bodies. They are usually deeper than 1.5 m and treat stormwater through sedimentation, detention, and nutrients uptake from the vegetation along the margin (Wong, 2006). According to some previous studies, when the hydraulic loading rate is 0.02 ~ 0.1 m/h, the removals of TSS, TN and TP are 47 ~ 80%, 22 ~ 25% and 19 ~ 51%, respectively (Mallin et al., 2002). In stormwater retention ponds, sedimentation and re-suspension mare the major processes for microbial removal (Pachepsky and Shelton, 2011). According to Krometis et al. (2009), the removal rates of *E. coli* in two stormwater retention ponds were 0% and 48% respectively; however, in some cases, an increase of pathogen indicators concentration in ponds has been reported (Davies and Bavor, 2000).

Previous sections discussed the performance of different types of WSUD systems in faecal microbial removal. A summary of indicator microbial removal rates in WSUD systems is provided in Table 2.4:

Table 2.4 Summary of indicator microbial removal rates in WSUD systems.

WSUD system	**Reference**	**Indicator microorganism**	
		E. coli	Faecal coliform
		Removal rate (%)	Removal rate (%)
Field-scale biofilters	Hathaway et al. (2009)	92	89
	Hathaway et al. (2011)	-119 ~ 70	-119 ~ 70
	Zinger et al. (2011)	96 ~ 99.98	99.26 ~ 99.98
	Chandrasena et al. (2016)	60.19 ~ 98.55	
Laboratory-scale biofilters	Zhang et al. (2010)	84	
	Zhang et al. (2011)	81.1 ~ 99.9	
	Li et al. (2012)	94.7 ~ 99.7	
	Chandrasena et al. (2014b)	> 73	
Wetlands	Díaz et al. (2010)	-554 ~ 95	
	Stenstrom and Carlander (2001)	99	
	Hathaway et al. (2009)	33 ~ 96	
Vegetated swales	CWSC (2010)		< 0
Stormwater retention ponds	Krometis et al. (2009)	0 and 48	
	Davies and Bavor (2000)	< 0	

Table 2.4 indicates that, compared to other WSUD systems, stormwater biofilters are the most promising system for stable in faecal microorganism removal, as they could achieve higher and more stable performance than other systems (e.g., wetlands). Therefore, the following literature reviews will mainly focus on stormwater biofilters. However, considering the inconsistency in microbial removal under field conditions, and the strict requirements in Australian guidelines (see Section 2.2.2), the optimisation of stormwater biofilters is necessary. To have a better understanding of how biofilters perform in microbial removal, the next section will discuss the major processes and governing factors of microbial removal in stormwater biofilters.

2.3.2 Stormwater biofilters

A typical biofilter (Figure 2.2) consists of filter media with a generally high sand content, a gravel layer at the bottom, and a sand transition layer in between (FAWB, 2009). Plants are cultivated on the top of filter media, and a small portion of water is normally kept in the gravel layer by elevating the outflow pipe to create a submerged zone (SZ).

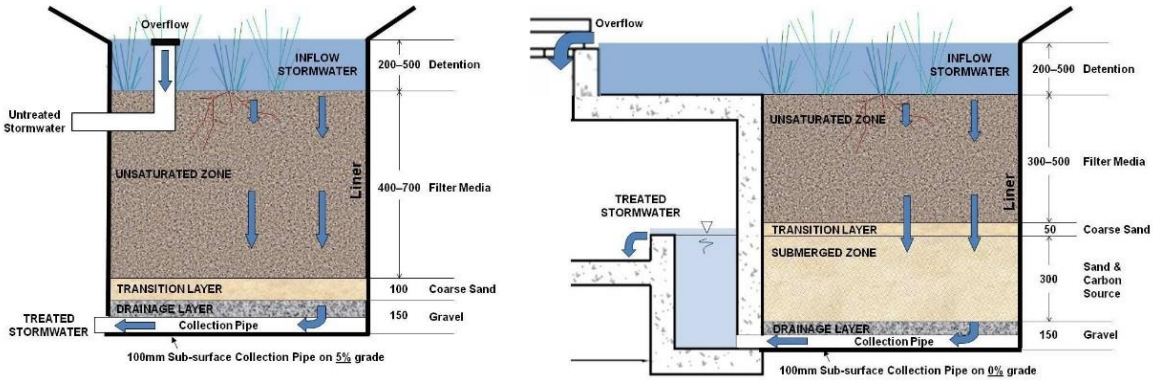


Figure 2.2 Schematic of typical stormwater biofilters with (the right figure) and without (the left figure) submerged zone (adopted from FAWB (2009)).

The importance of SZ was revealed by many studies, and the inclusion of SZ is highly recommended under any possible situation (Chandrasena et al., 2014b; FAWB, 2009). There are at least three functions of SZ: (1) the existence of SZ could decrease hydraulic head to increase retention times; under the same hydraulic conductivity, a biofilter column without SZ have a 60% higher infiltration rate compared to that with SZ; therefore, the biofilters with SZ could provide more retention time and accordingly lead to better removal performance (Chandrasena et al., 2014b); (2) SZ water after dry days is normally with high quality and could make contributions to dilute the final composite outflow; SZ could retain water from previous events; this water has a high retention time as it stays in SZ for the whole dry period between rainfall events; therefore, SZ water will experience enhanced removal by different processes; as a result, the retained water could be of low concentration of pollutants; during the subsequent wet weather events, this retained water could contribute to a lower composite pollutant concentration by diluting the newly treated water (which has shorter retention time) (Chandrasena et al., 2014b); and (3) the water

retained in SZ could keep plants survive during extended dry weather periods by providing sufficient water for plant uptake (FAWB, 2009; Payne et al., 2014).

Biofilters have been employed for the removal of total suspended solids, nitrogen, phosphorous, heavy metals, microorganisms, and micropollutants (Bratieres et al., 2008; Chandrasena et al., 2017; Chandrasena et al., 2016; Feng et al., 2012; Fowdar et al., 2017; Hathaway et al., 2011; Hatt et al., 2007; Li et al., 2016). It should be noted that, for different target pollutants, the design of a certain part of biofilters might be different. The key design features of stormwater biofilters are listed in Table 2.5.

Table 2.5 Key design features of stormwater biofilters (adopted from FAWB (2009) and Chandrasena (2014)).

Design	Criteria
Filter media	
Composition	High sand content with < 3 % clay and silt fraction to maintain structural stability and permeability (FAWB, 2009). Total nitrogen < 1000 mg/kg, $PO4^{-3}$ < 80 mg/kg for high to avoid leaching of nitrogen and phosphorous from the media (FAWB, 2009). Higher organic content for heavy metal removal (Feng et al., 2012).
Depth	Minimum of 300 mm to support plant growth and heavy metal removal (Feng et al., 2012); normally 400 ~ 600 mm (FAWB, 2009).
Hydraulic conductivity	100 ~ 300 mm/hour (FAWB, 2009).
Surface area	At least 2 % of catchment imperviousness for high nutrient removal (Bratieres et al., 2008). At least 4 % of catchment imperviousness for high heavy metal removal (Feng et al., 2012).
Submerged zone	
Composition	Medium to coarse sand or fine gravel with a mixture of carbon source (mulch/woodchips) (10 % by volume) to promote denitrification (Bratieres et al., 2008; FAWB, 2009).
Depth	Minimum of 300 mm to be effective (FAWB, 2009). 450 mm is optimal for nitrogen removal (Zinger et al., 2013).
Vegetation	*Carex appressa* for both high nutrient and heavy metal removal (Bratieres et al., 2008; Feng et al., 2012). *Melaleuca ericifolia, Goodemia ovate, Ficinia nodosa, Juncus amabilis, Juncus flavidus* for high nutrient removal (FAWB, 2009). *L. continentale*, *M. incana* and *Palmetto Soft Leaf buffalo* for *E. coli* removal (Chandrasena et al., 2014b).

In addition to design, the maintenance of biofilters is also crucial to ensure the effectiveness of a system. Many studies and practical experiences have reported that lacking of maintenance (e.g., clogging) could lead to the failure of biofilters (Brown and Hunt, 2012; FAWB, 2009).

Regarding to microbial removal, at least three types of weather conditions/rainfall events could negatively impact biofilters' performance: (1)

events with a large amount of inflow volume, as excessive inflow will exceed the treatment capacity of biofilters – after biofilters reach to the breakthrough point, the removal efficiency will gradually decrease (Chandrasena et al., 2014b); (2) events with long antecedent dry period (e.g., ≥ 14 days), as extensive dry days between two rainfall events will result in the wilt or death of plants, and accordingly reduce the plants' effects in microbial removal such as adsorption (Li et al., 2012); in addition, after plants consume most of the water kept in submerged zone during dry days for survival, the "clean" submerged zone water that experienced long-time die-off is not of a sufficient volume to dilute the newly entered water (which is less clean due to insufficient die-off), hence the composite outflow in current event is of high concentration (Chandrasena et al., 2014b); (3) events with short antecedent dry period – if a new rainfall event occurs after only a short dry period, the microbes attached onto the filter media from the previous event could not experience sufficient die-off and will be flushed off after remobilisation (Chandrasena et al., 2012); in addition, the submerged zone water is also not clean enough to dilute the newly entered water due to the insufficient retention time.

In summary, the consistency of microbial removal in stormwater biofilters need to be improved by enabling biofilters adjustable to those extreme conditions.

2.4 Governing processes and factors for microbial removal in stormwater biofilters

Adsorption, desorption, straining, and die-off are reported as the major processes for faecal microbial removal in stormwater biofilters (Bradford et al., 2006; Hathaway et al., 2011; Li et al., 2012; Zhang et al., 2011). In the meantime, these processes are governed by various design and operational factors. The major processes and the corresponding governing factors for microbial removal in stormwater biofilters is summarised in Figure 2.3. In this section, the theories of these processes, and how design and operational factors govern these processes, will be discussed.

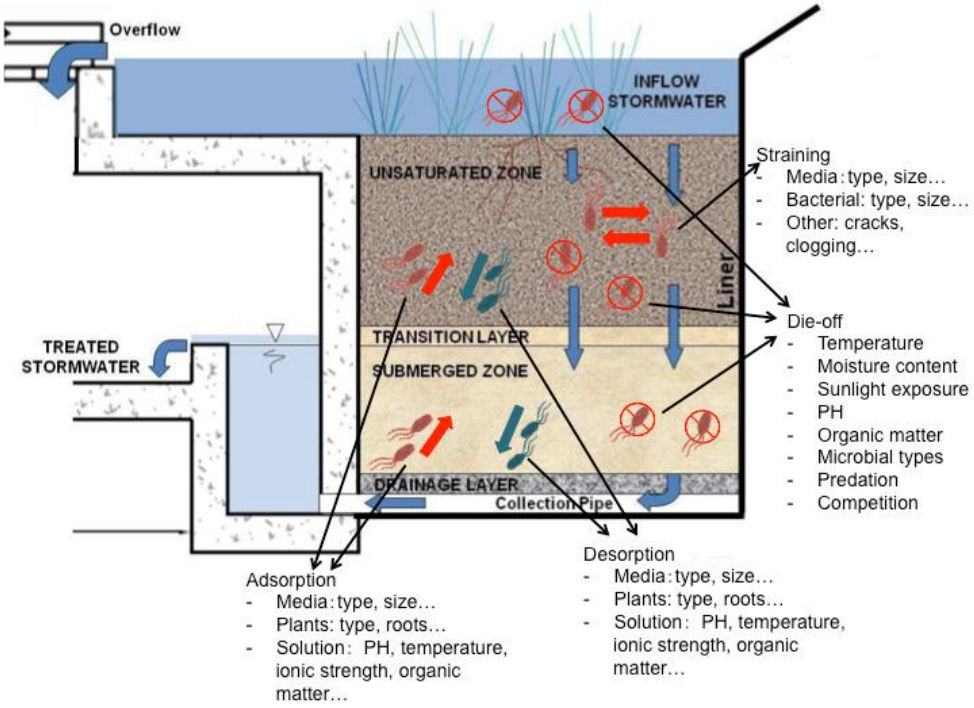


Figure 2.3 Major processes and corresponding governing factors for microbial removal in stormwater biofilters.

2.4.1 Adsorption

Adsorption is one of the most important governing processes for faecal microorganisms removal in stormwater biofilters (Chandrasena et al., 2013; Hathaway et al., 2011; Li et al., 2012; Zhang et al., 2010), and it is the dominant mechanism in porous media when the pores are larger than the bacteria (Stevik et al., 2004). It was explained by the double layer theory, which describes the repulsive and attractive forces between the bacteria and the substratum (Stevik et al., 2004). Adsorption is influenced by many conditions, such as media characteristics, plants characteristics, and solution and flow conditions.

(1) Media characteristics

A good adsorption effect could be achieved when the media size is small, as smaller media size result in larger surface area per unit volume to be provided for adhesion. In contrast, if filter media has macropores, the adsorption effect would be reduced due to insufficient contact time and increased distance between the microbes and the filter media (Stevik et al., 2004). It should be noted that, the media size or the pore spaces between media may change

during operation. For example, after a long time operation of biofilters, clogging may occur due to the accumulation of stable solid materials between or on the surface of media, and hence the adsorption would be enhanced (Kandra et al., 2014). However, during a long dry period, cracks may occur, which could result in a diminished microbial removal effect in the next rainfall event (Li et al., 2012).

Media type also governs the adsorption effect due to different cation exchange capacity: clay particles could improve adhesion due to their small size, platy shapes, and their large cation exchange capacity (Stevik et al., 2004). In addition, adsorption could be enhanced in the solid surfaces that are positively charged due to aluminium, iron, or manganese oxide coatings and *in situ* precipitation of metallic salts (Zhang et al., 2010).

Furthermore, the presence of biofilms in the filter media could reinforce adsorption (Stevik et al., 2004). This effect was found to be more effective in matured stormwater biofilters (i.e., biofilters that have been operated after a long time) than in newly established ones (Zhang et al., 2011).

(2) Plants characteristics

The presence of plants may have both positive and negative effects for adsorption. In some studies, a large amount of microorganisms near plant roots were reported, which indicated that plant roots might provide additional adsorption sites and an environment preferred by some microorganisms (Mukerji et al., 2006). However, long and thick plant roots may also cause macropores, and therefore the adhesion sites are reduced (Rusciano and Obropta, 2007); in addition, the presence of plant roots may also cause preferential flow paths, which would reduce the contact time and increase flow rates, so that the adsorption effect would be attenuated (Hathaway et al., 2011). Therefore, a comprehensive evaluation of these effects should be considered when selecting plants for biofilters.

(3) Solution and flow conditions

pH is a major factor for adsorption in solution conditions, especially for the adsorption of virus (Ferguson et al., 2003). Adsorption is reversible in pH range

of 7 ~ 8 (Schijven and Hassanizadeh, 2000). Since the pH of stormwater was reported within the neutral pH range (Duncan, 1999; NHMRC, 2009), microbial adsorption in biofilters would be reversible, and desorption may occur. The ionic strength of solution is another major factor: higher ionic strength of the carrying solution reduces repulsive forces, and adsorption is therefore reinforced (Stevik et al., 2004). Furthermore, the presence of dissolved organic matter could decrease the attachment of pathogens to the soil because adsorbed organic matter increases hydrophobic binding sites (Schijven and Hassanizadeh, 2000). Considering the various nutrients contained in stormwater, the concentration of organic matters would be an important factor for microbial removal in biofilters. In addition, adsorption could be enhanced in higher temperatures (Stevik et al., 2004). During the long time operation, the weather conditions may fluctuate frequently; hence, temperature is also a crucial factor that should be considered for microbial removal in stormwater biofilters.

Infiltration rate is also an extremely important factor, as it determines the treatment time of microbial in biofilters. Low infiltration rate has proved to be favourable for microbial removal, because lower infiltration rate provides longer time for all the governing processes to take place (not only for adsorption but also for other processes that will be discussed later). Therefore, high flow rates and preferential flow caused by macropores would result in the diminishment of microbial adsorption (Stevik et al., 2004). It is noted that, the infiltration rate is determined not only by inflow rate, but also the size and shape of plant roots, the inherent characteristics of filter media (e.g., media type and size), and cracks and clogging (Coustumer et al., 2012).

2.4.2 Desorption

As mentioned in Section 2.4.1, desorption of microbes normally occurs after adsorption due to the neutral pH of stormwater. The presence of microbial concentration tailing in the outflow breakthrough curves demonstrated the contribution of desorption in subsurface transport (Bales et al., 1991; Harter et al., 2000). It has been found that the changes of physical and chemical characteristics in the solutions could result in desorption: Bales et al. (1991) reported an increase in bacteriophage desorption from a silica beads filter

media when the pH of solution was increased; Redman et al. (2004) found that using a carrying solution with low ionic strength could increase the *E. coli* desorption from quartz sand. In addition, the presence of organic matter in the carrying solution might decrease adsorption and increase desorption (Franchi and O'Melia, 2003). Furthermore, microbes in fresh carrying solution could also lead to desorption due to their hydrodynamic interaction with previous retained microorganisms, especially under high flow rates (Tong and Johnson, 2006). Therefore, for faecal microbial removal in biofilters, the water quality of inflow and inflow rate are extremely important and dominate the effect of desorption.

2.4.3 Straining

Straining is a physical process that the microbe are trapped by pore sizes that are smaller than the microbial size. Therefore, the size and shape of microbes and filter media, the presence of cracks and macropores, and the clogging of filter media play an important role in straining (Ferguson et al., 2003; Stevik et al., 2004). Indeed, when the average cell size of microbes is greater than the size of 5% of filter media, straining becomes an important removal mechanism (Stevik et al., 2004). The presence of macropores in filter media cause substantial microbial movement and reduce straining, whilst the microbes with larger sizes are easier to be strained (Stevik et al., 2004); similarly, the clogging of filter media increases the straining effectiveness (Bright et al., 2010).

Although several studies have reported the importance of straining for microbial removal in porous media (e.g., as per Bradford et al. (2006) and Gargiulo et al. (2007)), whether these findings could be transferred to stormwater biofilters is questionable. Chandrasena et al. (2014a) indicated that in stormwater biofilters, straining is only important in the top sediment (i.e., a few centimetres of accumulated sediment layer that on the top of biofilters). Actually, microorganisms in biofilters exist either as free entities with a size that mostly ranges from 0.2 to 2 µm (Tortora et al., 1998), or associated with fine particulate matter (e.g. < 6 µm according to Brown et al. (2013)); compared to these sizes, the 5th percentile media size in biofilters could be much greater (e.g, > 50 µm as per FAWB (2009)). As Updegraff (1983) reported that straining only becomes an important removal mechanism when the average microbial size is

greater than the 5th percentile media size, the effect of straining might be negligible in some circumstances.

2.4.4 Die-off

While adsorption, desorption and straining are the main processes for microbial capture during wet weather, die-off is the process that takes place in both wet and dry weather (Chandrasena et al., 2013; Chandrasena et al., 2014a). More importantly, die-off is the most crucial process that governs microbial survival during dry days (Chandrasena et al., 2014a; Zhang et al., 2011). The effect of die-off is influenced by two types of conditions: abiotic conditions and biotic conditions. Abiotic conditions include temperature, moisture content, sunlight exposure, pH, organic matter and salinity; whist biotic conditions include microorganism spices, predation and competition.

(1) Temperature

Temperature is reported as one of the most significant factors that govern microbial die-off (Bitton and Gerba, 1984; Chandrasena et al., 2014a; Ferguson et al., 2003; Schijven and Hassanizadeh, 2000). Generally, die-off rate increases with the increase of temperature: Chandrasena et al. (2014a) tested the die-off rates of *E. coli* under 2.5, 15 and 32.5°C, and found that the *E. coli* concentration increased over time under 15 or 32.5°C, and decreased when the average incubation temperature is 2.5°C; in addition, Chandrasena et al. (2014a) reported that the *in situ* average *E. coli* die-off rate increased from 1.32 day^{-1} to 2.58 day^{-1} when the average incubation temperature increased from 15 to 32.5°C. Similarly, Zhang et al. (2012) found that the *E. coli* die-off rate increased from 0.11 to 1.87 per day when the temperature in the conventional filter media increased from 5 to 37°C. In addition, Vandenhove et al. (1991) found that for the die-off rates of *Pseudomonas sp.* in soil, there was no difference between 5 and 15°C, but a significant increment at 25°C.

Importantly, Zhang et al. (2012) found that the microbial growth rate decreased when the temperature increased from 25 to 37°C. However, these protozoa were native in the biofilter rather than introduced by newly entered stormwater. Therefore, the effect of temperature on die-off would be various for different

types of microorganisms. Moreover, Garzio-Hadzick et al. (2010) found that the increase of nutrient contents and fine particles resulted in a decrease of the sensitivity of *E. coli* survival towards the soil temperature. This finding indicated that different governing factors might interact with each other.

Furthermore, in stormwater biofilters, various temperatures have already been detected in different locations and under different weather conditions. For example, Hathaway et al. (2011) reported that the soil temperature in stormwater biofilters could vary from 7 to 34 °C. In addition, the effect of temperature on microbial survival in biofilters would be more prominent in upper soil layers compared to deeper soil layers, as upper layers undergo relatively large, daily temperature variations (Jones and Hunt, 2009).

(2) Moisture content

Moisture content (water potential) is another factor that reported crucial to microbial die-off, especially for bacteria (Bitton and Gerba, 1984). Generally, under lower water potential the stress on microorganisms in increased because of the increased effects of matric and osmotic potentials (Ferguson et al., 2003). Therefore, the majority of literatures suggested that the bacterial survival was diminished in dry soils compared to that observed in moist soils (Stevik et al., 2004; Yates and Yates, 1987). Accordingly, the lower moisture content may result in better microbial removal in biofilters.

Nonetheless, some studies reported the opposite results: as per Bitton and Gerba (1984), there was a better protection from predators in dry soils, leading to a longer microbial survival. Zhang et al. (2011) also suggested a logarithmic decrease of trapped *E. coli* with increasing depth in biofilter columns, where a steady increase in soil moisture content was observed and might result in increased predator mobility.

(3) Other abiotic conditions

Other abiotic conditions include sunlight exposure, pH, and organic matter. It is well documented that sunlight could induce pathogens die-off (Brookes et al., 2004; Chandrasena et al., 2014a; Ferguson et al., 2003; Hipsey et al., 2008).

According to Brookes *et al.* (2004), solar radiation mainly causes two types of damage to microorganisms: photo-biological (direct damage induced UV bandwidth) and photo-oxidative (indirect damage caused by the visible bandwidth of incoming solar radiation). However, it should be noted that, in biofilters, the effect of radiation would only be significant in the topmost part of the system (e.g., in the ponding zone and top sediments), sunlight could hardly penetrate into deeper soil layers and might be blocked by vegetation. Chandrasena et al. (2014a) reported that the exposure to sunlight is one of the most important factors for the *E. coli* retained in the top sediments.

The survival of most bacteria decreases with both low and high pH values, while the survival may be prolonged at neutral pH values (Stevik et al., 2004; Yates and Yates, 1987). However, the recorded pH values for both stormwater (Duncan, 1999; NHMRC, 2009) and biofilter media (FAWB, 2009; Hathaway et al., 2011) are in the neutral range. Therefore, pH would not play an important role in microbial die-off in stormwater biofilters.

It has been reported that pathogens could survive longer in soils containing organic matter (Stevik et al., 2004) and even grow with sufficient amounts of organic matter (Yates and Yates, 1987). The reason is, high levels of organic matter could provide essential nutrients for microbial metabolism, and attenuate the effects of competition for nutrients with indigenous microorganisms (Crane and Moore, 1986; Willey et al., 2011). In stormwater biofilters, filter media generally contains low organic matter content; therefore, the organic matter mainly depends on inflow (FAWB, 2009). To improve this, loamy sand filter media (with relatively high fine fraction) was applied in some studies, and it is proved to be more favourable for microbial survival compared with pure sand filter media due to its high efficiency in retaining nutrients and organic matter (Bratieres et al., 2008). The organic matter content is especially important during dry periods when no new organic matter is introduced and the decrease of nutrients occurs through the consumption by both enteric and indigenous microbial populations (Chandrasena et al., 2014a).

To summarise, in stormwater biofilters, the organic matters contended in inflow are crucial for microbial removal, whilst sunlight exposure and pH are not playing important roles.

(4) Microorganism types

The survival of microorganisms differs between types. Compared to *E. coli*, several *Salmonella* and *Yersinia* species have longer survival times, whilst *Shigella* and *Campylobacter* die more rapidly (Stevik et al., 2004). In addition, some *Streptococcus* species could survive much longer than others (Stevik et al., 2004). The reason could be, some bacteria may have better ability to compete for nutrients with indigenous microorganisms, some may be less vulnerable to antibiotics produced by indigenous bacteria, and some others can produce spores to improve survival under unfavourable conditions (Stevik et al., 2004). In addition, Schijven and Hassanizadeh (2000) indicated that the sensitivities to temperature were varied between different virus types; hence, the change of abiotic conditions may have different influences to different types of virus. However, according to Chandrasena et al. (2014a), the survival patterns of different strains of *E. coli* are generally similar.

(5) Predation and competition

The presence of other microorganisms plays a vital role in the survival of captured microbes in the biofilters. For example, Chandrasena et al. (2014a) reported that a steady decline in *E. coli* concentration was observed in the samples collected from different locations of biofilters, but a general trend of increasing concentration of *E. coli* was observed in sterilized soil samples. The presence of other microorganisms may have two types of impacts to faecal microbial removal: predation and competition.

From the predation perspective, protozoa are considered as the main predators of bacteria (Stevik et al., 2004). They are also the major contributors to *E. coli* die-off within stormwater biofilters (Zhang et al., 2010; Zhang et al., 2011; Zhang et al., 2012). In laboratory-scale stormwater biofilters, *E. coli* only grow in the absence of other microorganisms (obtained by γ-irradiating the filter media); even a small increase of protozoa into the γ-irradiated filter media could

change the growth into die-off (Zhang et al., 2010). The authors also found that when the protozoa levels were increased by just one order of magnitude, a 30 % increase in the *E. coli* die-off rate was observed. In a later study conducted by the same authors (Zhang et al., 2011), it was found that with the maturation of indigenous predator protozoa's population, the effect of predation on *E. coli* die-off was reinforced.

Importantly, it is reported that bacteria might attempt to avoid predation by aggregation and attaching to other particles (Ferguson et al., 2003). This is because for large predators such as protozoa, it is difficult to locate their prey when they are situated in small pores of soil and sediment (Alexander, 1981).

From the competition perspective, microorganisms require nutrients to support their metabolic activities and subsequent growth (Willey et al., 2011). Indigenous microorganisms in natural environment fulfil their nutrient requirements from organic matters in soils and/or water; when enteric microorganisms are released into natural environment, they have to compete with indigenous microorganism for nutrients, because generally the natural environment contains lower level of nutrients compared to enteric microorganisms' primary habitats like guts of warm-blooded animals (Alexander, 1981; Burton et al., 1987; Lim and Flint, 1989). However, it is reported that enteric microorganisms are unable to adequately compete with indigenous microorganisms; therefore, competition is also considered as a factor that contributes to the elimination of enteric species introduced into water or soil (Alexander, 1981; Burton et al., 1987; Lim and Flint, 1989).

In stormwater biofilters, the competition for nutrient with indigenous bacteria is a factor for *E. coli* survival (Zhang et al., 2010; Zhang et al., 2011; Zhang et al., 2012). However, Zhang et al. (2010) observe that *E. coli* die-off increases with increasing indigenous protozoa levels in two laboratory biofilter columns with similar levels of heterotrophic bacteria. Therefore, they concluded that the significance of competition might be less compared to predation. Nevertheless, in another laboratory experiments on biofilter columns, Zhang *et al.* (2012) found that competition can be significant at a high temperature (e.g. 37°C),

because with this temperature, the predator protozoa growth is limited due to encystment, but this condition is optimum for enteric bacterial growth.

2.4.5 Summary of governing processes and key factors

To summarise, for faecal microbes removal in stormwater biofilters, adsorption, desorption, straining and die-off are the governing processes. Adsorption, desorption and straining play a vital role during wet weather events, whilst die-off is dominant in dry periods between rainfall events.

In the meantime, these governing processes are highly dependent on the factors/conditions in biofilters. These key factors could be divided into types: design factors, operational factors, and other factors (Table 2.6).

The most important design factors include media size, media type, plants type, the existence and depth of SZ, while the key operational factors include temperature, moisture content, and infiltration rate (Table 2.6). The effects of these two types of factors have been discussed in previous sections.

For other factors, weather conditions and inflow characteristics are the most crucial ones (Table 2.6). For weather conditions, the duration and intensity of a rainfall and the length of dry period between two wet events are significant, since the duration of rainfall determines the hydraulic loading, whilst the length of dry period may have impact to the survival of plants and have potential to cause cracks (Zinger et al., 2013). For inflow characteristics, the inflow quantity and inflow quality are both important. For hydraulic loading, Le Coustumer et al. (2012) indicated that larger inflow quantity could cause clogging more easily, and clogging could improve infiltration rate and may also result in more overflow. For inflow quality, not only the *E. coli* concentration but also the concentrations of organic matters and total suspended solids (TSS) are important, since organic matters could influence adsorption as well as die-off, and TSS concentration may influence the extent of clogging.

Table 2.6 Summary of key factors for microbial removal in stormwater biofilters and how some of these factors influence microbial removal processes. "↑" stands for "increase", "↓" means "decrease", while "–" represents "result in".

	Design factors		Operational factors		Other factors
Media	Size (↓) – adsorption (↑) & straining (↑); cation exchange capacity (↑) – adsorption (↑); organic matters (↑) – die-off (↓)	**Temperature**	Temperature (↑) – adsorption (↑) & die-off (↑)	**Weather**	Event size; length of dry periods
Plant	Size of roots (↑) – adsorption (↑ or ↓)	**Moisture content**	Moisture content (↓) – straining (↑) & die-off (↑)	**Inflow**	Inflow rate; water quality
SZ	The existence of SZ – infiltration rate (↓) & retention time (↑)	**Infiltration rate**	infiltration rate (↓) – retention time (↑)		

To enhance microbial removal in stormwater biofilters, much effort has been placed in optimising the design factors of biofilters, such as testing the influences of different plants, various filter media, and the existence of a submerged zone (Chandrasena et al., 2014b; Li et al., 2016; Li et al., 2012). Some of the findings have already incorporated into guidelines of biofilters design (FAWB, 2009). However, the optimisation of design factors could hardly mitigate impact of some extreme weather conditions, such as extended dry periods and big size events (Chandrasena et al., 2014b; Li et al., 2012).

However very little has been done to control the operational conditions experienced by biofilters (e.g., infiltration rate and retention time), even though they are recognised as governing microbial removal (Chandrasena et al., 2014a) and would be a solution to enable biofilters adapt to various weather conditions. That is because, stormwater biofilters are designed as passive treatment systems, and there are no facilities on biofilters to *control* their operation. Therefore, it is important to find a method to better control biofilters in operational. For instance, if there is a method to transform biofilters from *passive* into *active* to achieve the operational optimisation, then the findings from previous studies could be taken into practice, and accordingly the performance in microbial removal of biofilters could be improved.

2.5 Real time control of stormwater biofilters

As discussed in Section 2.4, the optimisation of operational conditions has hardly been taken into practice, since biofilters are designed as a passive treatment system. To solve this problem, real time control (RTC) is a potential technology to transform biofilters from passive to active, and enable biofilters adjustable to various weather patterns and inflow conditions.

Very few studies on the application of RTC in stormwater biofilters, if there is any, have been published. Therefore, this section will mainly discuss the application of RTC in other environmental areas (e.g., flood control, wastewater treatment, and sewer systems), to analyse the potential of applying RTC in stormwater biofilters.

2.5.1 General concept and components of RTC

The general concept of RTC is defined as the monitoring of the functioning of a system in real time and, at the same time, using the collected data to optimise system's performance through the control (Schütze et al., 2004).

The monitoring and control of a system are implemented by hardware components, including sensors (e.g. temperature sensors and moisture sensors; to monitor system status), actuators (e.g. pumps, valves, and water gates; to implement control), controllers (e.g. programmable logic controllers; to adjust actuators to achieve its desired set-point), and data transmission systems (to transmit data between the different devices) (Schütze et al., 2004). The process of control can be schematised as a control loop, which is shown in Figure 2.4 (Schütze et al., 2004):

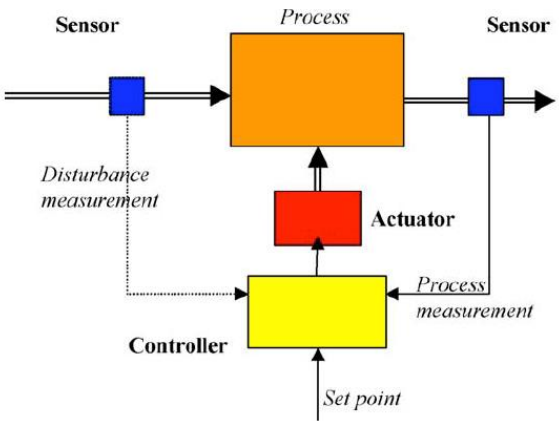


Figure 2.4 Feed-forward (disturbance measurement) and feedback (process measurement) control loop of RTC. Simple arrows indicate data flow, double arrows indicate hydrodynamic action. Bold letters indicate hardware and italic letters indicate variables (adopted from Schütze et al. (2004)).

Normally, the first step of developing a RTC system is to establish a model to simulate the key process variables, as the model could serve as a tool to predict the outcome of control actions (Schilling et al., 1996). In addition, a control strategy is necessary in a RTC system, as it governs the timing of control and the set-points of actuators (Schilling et al., 1996; Schütze et al., 2004). The control strategies could be in the form of decision matrices, decision trees (with a set of if-then-else rules), or optimisation routines (Schilling et al., 1996; Schütze et al., 2004). The model must allow for the input of control strategies and predict the outcome of strategy implementation.

Mathematical optimisation algorithms have been widely employed to figure out suitable control strategies; the most popular mathematical optimisation algorithms include non-linear programming algorithm, genetic algorithm, neural networks algorithm (Schilling et al., 1996; Schütze et al., 2004). However, Schütze et al. (2004) suggested that complex control algorithms are not always necessary, since an intelligent and supervised combination of simple *single input - single output* control laws could also be effective and easy to implement.

It is noted that, multi-objective optimisation is often necessary, in a practical problem, objectives are normally multiple rather than single. For example, in

the majority cases in the environmental field, water quality and water quantity should both be taken into consideration; in addition, the cost-effectiveness is also a non-negligible criterion when evaluating technical solutions.

In addition, when designing a RTC system, several upmost important requirements should be met, including safety (i.e., the worst-case scenario in RTC implementation should be no worse than the situation without RTC), adaptability (i.e., RTC equipment could provide reliable measurements and data processing), flexibility (i.e., system could adapt to various conditions, including different weather patterns and equipment failures), and acceptable cost-effectiveness (Schütze et al., 2004).

2.5.2 Application of RTC in the environmental field

Although currently publications about RTC of stormwater biofilters could be hardly found (if there is any), RTC has been applied in some other areas in the environmental field, such as flood control, sewer systems, and wastewater treatment. The purposes of these applications include to route flows, to control flooding and overflows, to maximise system's storage space, to optimise system's treatment capacity, and to protect the quality of receiving waterbodies.

(1) Flood control

Chang and Chang (2001) developed an approach with the combination of the genetic algorithm (GA) and the adaptive network-based fuzzy inference system (ANFIS) to improve real time flood-reservoir operation. GA was used to search the optimal reservoir operating histogram based on the inflow series, and then ANFIS was used to construct the suitable structure and parameters, and to estimate the optimal water release according to the reservoir depth and inflow conditions. According to the operation of the Shihmen reservoir in Taiwan, the authors indicated that the RTC approach had a superior performance regarding the prediction of total water deficit and generalized shortage index.

Similarly, in some other cases, sophisticated models and control strategies were developed for RTC. For example, Leon et al. (2014) introduced a RTC framework based on multi-objective non-dominated sorting genetic algorithm II

based on reservoir operation. Hsu et al. (2015) applied an adaptive network-based fuzzy inference system incorporated real time recurrent learning neural network in reservoir optimisation. Tian et al. (2015) employed model predictive control (MPC) to operate the structures like dams, dikes and water gates in Rhine - Meuse delta. All these studies indicated that fruitful benefits could be obtained by the implementation of RTC.

The examples introduced above suggested that the integration of adopting predictive models and implementing control strategies with various complexity could achieve great benefits in RTC, which could provide lessons in developing a framework for RTC of stormwater biofilters. In addition, a sophisticated RTC strategy could multi-objective optimisation.

(2) Sewer systems

The application of RTC in sewer systems started in the late 1960s in the USA with implementations in Seattle, Cleveland, Detroit, Minneapolis, etc. (Schilling et al., 1996). About 10 years later, the first European system was implemented; nowadays, most of the RTC implementation are conducting in the Netherlands and Germany (Schilling et al., 1996). The most typical and successful RTC systems are outlined as follows.

In the Lundofte catchment in Denmark, where a costly expansion of traditional infrastructure is planned, a rule-based RTC strategy was implemented to increase storage volume of urban drainage systems (Meneses et al., 2018). After implementing RTC, the planned storage volume was reduced by 21%, while the combined sewer overflow (CSO) volumes, environmental impacts, and utility costs, were all reduced by up to 10%.

Seattle public utilities operated a predictive RTC system to manage 13,120 acres combined sewers to optimise flow by adjusting the gate position for each of the 15 sewer regulators. With RTC, overflow volume was reduced by 60 ~ 90 % and water quality was also controlled (Stinson, 2005).

Milwaukee Metropolitan Sewerage District has been operating a RTC system for the control of deep tunnels with a 400 million gallon capacity more than 20 years (Schultz et al., 2001). The tunnel intercepted both combined and sanitary sewer, and remarkable cost-effective savings on storage space volume were achieved (Stinson, 2005).

The Urban Drainage Network of Seine St.-Denis County in France has been operating a RTC system for sewer systems since 1986. The main objective is to reduce combined and separated sewer systems to the Seine River. By controlling six hydraulic overflow regulators include a settling and storage facility of 52.8 million gallons, the settling efficiency and the sewer capacity were increased. The comparison of the performance with two control types (i.e., supervisory global predictive control with a radar-based rainfall prediction system, and a simulated local reactive control) indicated that the global predictive control performed better, particularly in suspended solids removal (Stinson, 2005).

The experiences learned from these studies that could be used to guide the RTC of stormwater biofilters include: it is crucial to determine the proper location of RTC facilities like sensors and regulators; for small-scale systems, it might be more effective to implement global predictive control than local optimisation; the integration of forecasting or now-casting technology for the weather information could be helpful to develop a RTC with high adaptability; and the cost-effectiveness of RTC systems is normally satisfying.

(3) Wastewater Treatment

To improve the performance of wastewater treatment, real time monitoring (RTM) and RTC are frequently used for the optimisation of key processes. The governing factors include pH, temperature oxidation-reduction potential (ORP), dissolved oxygen (DO), and water level are usually monitored and controlled as well (Han and Qiao, 2014; Schütze et al., 2004; Yang et al., 2007). Some typical cases of RTC application in wastewater treatment plant (WWTP) are outlined below.

Yang et al. (2007) applied RTC in the sequencing batch reactor (SBR) of a WWTP to save energy and carbon source, and to achieve advanced nitrogen removal. By controlling the temperature ranging from 11.9 to 26.5 °C under normal dissolved oxygen condition (2.5 mg/L), nitrogen removal via nitrite was successfully and stably achieved for a long period (180 days) with average nitrite accumulation rate above 95%. In addition, *in situ* fluorescence hybridisation results approved that the nitrifying microbial communities were also optimised.

Han and Qiao (2014) discussed the design and implementation of a non-linear model predictive control (NMPC) system for WWTP operation. A hydraulic model and a biochemical model were integrated and utilised to control DO and nitrate concentrations. Experimental results revealed that the proposed RTM and RTC provided satisfactory tracking of DO and nitrate, and attenuated the fluctuation in treatment effects.

Corominas et al. (2006) used an on-line control strategy to optimise the operation of the SBR for a WWTP. A calibrated mechanistic model based on the Activated Sludge Model No.1 was used to evaluate the control strategy; the ORP and DO were monitored to optimise the aerobic and anoxic phases. The performance was evaluated with the criteria of the effluent quality, the required energy for aeration, and the treated wastewater volume. The results showed that by adopting RTC, it was possible to maintain optimal SBR performance with minimal costs.

For RTC in stormwater biofilters, it could be learned from these successful cases that, when using a predictive model to evaluate RTC strategies, the model may include a hydraulic part and a reaction part (for water quality). In addition, it is feasible and reliable to monitor some operational factors (e.g., DO concentration, temperature, and ORP) and using this information to trigger control and keep a system's performance in a consistently high level.

(4) Combination of sewer systems and wastewater treatment plants

Since sewer systems and wastewater treatment plants are usually connected, sometimes RTC is implemented to control the integration of these two systems.

Schütze et al. (2004) described a wastewater system with RTC of the Québec Urban Community, which consists of sewer networks, a wastewater treatment plant (WWTP) and retention tunnels. The control objectives include to minimize overflows and to maximize the use of the treatment plant capacity. According to the need for local or global optimisation, three control levels were set. The real time control system is implemented at a central station, based on the information of water level, rainfall intensity, radar rainfall images, and two hours rainfall prediction that obtained from 17 flow monitoring and weather stations. A non-linear programming algorithm was used for optimisation, which ran every five minutes as a time step. The performance of this RTC system was remarkable: by only optimising two tunnels and the capacity of the WWTP, a 70% reduction in overflow volume in 2000 was achieved; in addition, the cost of this phase was 2.6 million US dollars compared to an estimated 15.5 million US dollars to build retention facilities to attain the same benefits.

Schilling et al. (1996) introduced the application of RTC in the Klagshamn wastewater system. To solve the problem of sludge loss from the secondary clarifiers in Klagshamn WWTP, a sewage system was used for wastewater storage to reduce flow variations. Sensors were set to detect the concentration of suspended solids in different treatment units, and the sludge blanket level in the secondary clarifier. A program package named MOUSE-PILOT was utilised to model the system. The authors concluded that approximately 50% of the total storage capacity of the system remained unused during wet weather if no control was implemented; with the application of RTC, about half of this potential might be activated.

Langeveld et al. (2013) analysed the potential of improving the Dommel River water quality using integrated RTC of WWTP and combined sewer systems. Four types of models were integrated: hydrodynamic sewer model, water quality model for sewer, WWTP model, and surface water model for receiving

water. The control objectives included minimising ammonium peaks in the river, and/or minimising dissolved oxygen dips in the river. The authors indicated that with the implementation of only simple and inexpensive RTC strategies, the number of exceedances for ammonium and DO decreased significantly.

These studies indicated that the performance of a system could be enhanced by even a short time step of optimisation, and a simple RTC strategy with generally low cost might already be sufficient to dramatically improve the treatment.

(5) Stormwater related application

Currently, stormwater related application of RTC mainly focuses on stormwater ponds or basins. For example, Mullapudi et al. (2018) applied the RTC network on two stormwater basins to shape streamflows. The developed strategy could stabilize flows, and accordingly mitigate the streambed erosion and reduce pollutant loads that discharged into waterbodies in the downstream. In addition, Gaborit et al. (2013) reported that by adopting RTC to monitor and adjust a stormwater pond's water height according to the rainfall forecast and detection, the total suspended solids removal efficiency could increase by 46 ~ 90 %. More importantly, Kerkez et al. (2016) introduced a concept of using cost-effective sensors and actuators to retrofit existing stormwater infrastructure in the watershed scale, exploring the conceptual potential of applying RTC in stormwater biofilters.

2.5.3 Summary and categorisation of RTC methods

In summary, all the successful cases about the application of RTC in other environmental areas that presented in Section 2.5.2 highlighted the remarkable benefits of RTC implementation, which provided strong encouragement and valuable lessons to the development of a framework for RTC of stormwater biofilters. Table 2.7 summarises all the methods employed in the studies discussed in Section 2.5.2 and some other relevant studies.

Table 2.7 Categorisation of RTC methods (adapted and summarised from Lund et al. (2018) and Schütze et al. (2002)).

Category	RTC method	Description
Degree of automation	Manual	Actuators are adjusted by operators.
	Supervisory	Actuators are automatically adjusted; set-points of the actuator are specified by operators or a supervisory system.
	Automatic	The entire system is automatically operated.
Physical extension	Local	Each actuator performs control independently based on instantaneous measurements.
	Global	All actuators are regulated at the same time, based on the observations from a global perspective.
RTC strategy	Off-line	Operational objectives are specified prior to the actual control process (e.g., in the form *if-then*); set-points are normally fixed.
	On-line	Operational objectives are defined as a mathematical function; set-points are variable and determined during the control process.
Timing of input	Reactive control	Control activities are only determined by measurements.
	Predictive control	Control activities are determined by measurements and the prediction of system's future status.

It is noted that, in the vast majority cases that discussed in Section 2.5.2, RTC was implemented in generally much larger scales compared to stormwater biofilters. Therefore, RTC in most of these cases integrated advanced facilities to achieve fully automatic control, and complicate algorithms to implement on-line strategies and fulfil global optimisation. Importantly, Schütze et al. (2004) pointed out that an intelligent and supervised combination of simple *single input - single output* control laws could also be effective and be implemented easily. In addition, complicated systems may have a lower likelihood to achieve cost-benefits as more facilities are required, and be less flexible facing to facility failure. All these experiences and lessons could be referred in developing a

framework for RTC in stormwater biofilters. The details of the framework development will be presented in the next section.

2.5.4 Framework for the application of RTC in stormwater biofilters

According to the aforementioned experiences and lessons learned from the application of RTM/RTC in other areas of the environmental field, and considering the characteristics of faecal microbes in stormwater runoff, the components in RTC implementation on stormwater biofilters are conceptualised in Figure 2.5.

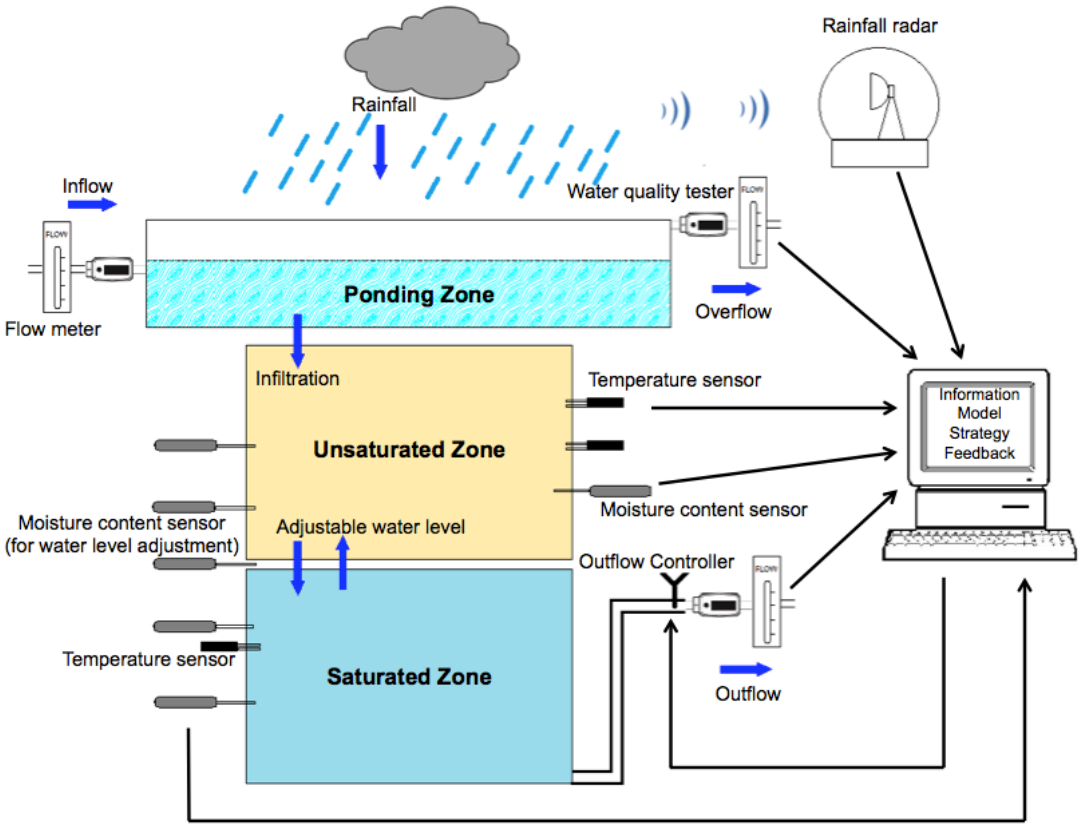


Figure 2.5 A conceptual schematic for RTC implementation on stormwater biofilters.

To implement RTC in stormwater biofilters for microbial removal, two types of components are necessary: software and hardware. For the software perspective, a predictive model to evaluate potential RTC strategies and to simulate different scenarios are indispensable; whilst for the hardware perspective, sensors to monitor different information in biofilters, actuators (e.g., valves and outflow restrictors), controllers (with set-points) and data

transmission systems are the essential components for RTC implementation. In addition, since the real time information of weather and/or rainfall forecasting could help to generate accurate and timely RTC strategies (Stinson, 2005), some advanced technologies such as radar or now-casting technology for rainfall prediction might also be incorporated.

One thing should also be noted that, for different end uses, different treatment objectives should be achieved; the treatment objectives govern the establishment of RTM/RTC facilities and selection of RTM/RTC strategies (i.e. what to monitor and what to control). For example, for the protection of waterbodies, the total amount of pollutants might be more important than other criteria; therefore, one of the criteria for control strategy selection is to ensure that, when the infiltration rate is decreased to increase treatment time, the extra pollutants reduction in outflow must surpass the additional pollution caused by additional overflow. While for recreational uses, the peak and/or 95th percentile concentrations should be controlled under an uncertain threshold. In addition, different strategies may aim to improve the removal of different pollutants; however, the better removal of one pollutant achieved by control strategies should not dramatically impact the removal of another pollutant being worse than the requirement.

It is also noted that, for RTC of biofilters, complicated algorithms for optimisation may not be necessary. Compared to the examples discussed in Section 2.5.2, the scale of biofilters is generally smaller, and the requirements for facilities like sensors and actuators would be lower. Therefore, it could be expected that even a simple RTC system with local optimisation might be sufficient to achieve the operational optimisation. In addition, considering the short duration of some rainfall events, stormwater biofilter with RTC are required to react rapidly to instantaneous inputs and biofilter status; since an off-line strategy normally needs less time to trigger control than an on-line strategy by avoiding to re-determine set-points (Lund et al., 2018), it could ensure RTC being implemented timely. Therefore, developing off-line strategies before the implementation of RTC might be favoured for stormwater biofilters.

Compared to testing all the potential RTC strategies one by one through laboratory experiments and/or field tests, modelling is more cost-effective to evaluate these strategies. As such, a predictive model for microbial removal stormwater biofilters serves as a fundamental part of RTC. The predictive model includes the governing processes and factors to sufficiently represent accurate microbial behaviours within biofilters; in addition, it should be capable of simulating different operational conditions and reflect how these conditions would impact the transport and fate of microbes. Moreover, considering the large number of potential strategies and scenarios, and the difficulty and uncertainty in data collection, the model that could generate accurate predictions with minimum data requirements and simulation time are favoured.

Based on all the analyses above, the conceptual framework for RTC of stormwater biofilters may include the following processes: (1) develop and validate a predictive model to simulate the microbial removal behaviours in stormwater biofilters; (2) develop potential RTC strategies to optimise the operation of stormwater biofilters; (3) select RTC strategies and implement them by integrating RTM, to analyse the benefits of RTC strategies and evaluate if/how RTC could help to fulfil different requirements (e.g., for different end uses); (4) employ predictive models to simulate the implementation of selected RTC, and compare the modelling results to those obtained from practical implementation, to evaluate the capability and reliability of using predictive model to evaluate RTC; (5) if the predictive model is proved to be a reliable tool to evaluate RTC strategies, additional RTC strategies and scenarios could be assessed through modelling, and a final decision of strategy selection and corresponding set-points could be made based on the modelling results (Figure 2.6).

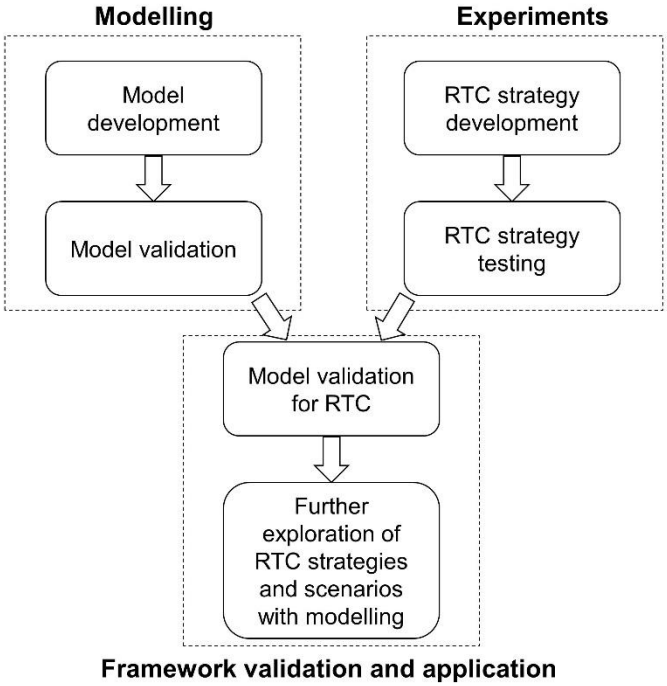


Figure 2.6 Schematic of the framework for real time control of stormwater biofilters.

Furthermore, according to the general requirements for a RTC system (i.e., safety, adaptability, flexibility, and cost-effectiveness; as listed in Section 2.5.1), the requirements for RTC of stormwater biofilters are specified as follows:

(1) safety: RTC is expected to deliver on both stormwater harvesting outcomes (i.e., high volume and good quality of the water for harvesting) and environmental protection (e.g., minimum pathogens for swimming, and low nutrients for ecosystem health).

(2) adaptability: the biofilters incorporated with RTC should be capable of adapting various conditions, such as different rainfall event sizes, different inflow concentrations, and different lengths of dry periods;

(3) flexibility: RTC strategies should be flexible in its implementation, allowing for easy system design and facility installation, both for new systems and for retrofit scenarios (meaning only surface accessible equipment can be installed); and

(4) cost-effectiveness: RTC that requires low maintenance and low-cost equipment (e.g., sensors) are favoured; hence, low-technical solutions are preferable.

As aforementioned, in this study, the main purpose of adopting RTC is for the optimisation of stormwater harvesting and reuse; therefore, faecal microbes are the major target pollutants in RTC implementation.

2.6 Predictive models for microbial removal in stormwater biofilters

Section 2.5 indicates that to apply RTC technologies in stormwater biofilters, a predictive model is essential for the evaluation of RTC strategies. Therefore, this section will discuss the existing models for faecal microbial removal in stormwater biofilters, and how to develop an effective predictive model for RTC of biofilters.

Generally, an effective predictive model for RTC should provide a better understanding of the governing processes, test different control strategies efficiently, and generate reliable predictions to help on decision making (Hipsey et al., 2008). To meet these requirements and according to the discussion in Section 2.5.2, an effective predictive model for faecal microbial removal in stormwater biofilters should be able to:

(1) represent the transport and fate of faecal microorganisms throughout biofilters;
(2) include the governing processes of microbial removal;
(3) reflect the influences of the key factors for microbial removal;
(4) represent both wet weather events and dry periods that between wet events; and
(5) generate accurate predictions with minimum data requirements and simulation time.

2.6.1 Existing models for microbial removal in stormwater biofilters

Only few attempts have been made to develop a model for microbial removal in stormwater biofilters. Chandrasena et al. (2013) developed a model to simulate the outflow concentrations continuously. In this model, adsorption and desorption were included as the major processes during wet weather periods, whilst die-off was considered to be dominated during dry days. Both adsorption

and die-off were described as first order kinetics. This model achieved a good level of agreement between the predicted and measured *E. coli* concentration in the outflow. The results also indicated that adsorption is the governing process in the model, and vegetation is found to have an impact on adsorption and desorption since the calibrated parameters vary significantly with the different plant configurations (Chandrasena et al., 2013). However, this model only modelled the outflow concentration and incapable of revealing the transport of microorganisms throughout the biofilters; in addition, the impact of operational factors was not reflected.

Zhang et al. (2010) utilised one-dimensional advection-dispersion equation to model the transport of *E. coli* in bioretention media during 6 hours of continuously simulated run-off conditions. This model also achieved a satisfying agreement between modelled and measured *E. coli* breakthrough curves. However, in this model only considered the processes of adsorption and die-off; desorption and straining were neglected. Also, this model did not reflect the influence of any operational factors. Moreover, the simulations were only limited to a single event rather than a long period of operation with several wet weather events and dry periods in between.

Zhang et al. (2012) used the first-order kinetics equation to model the die-off process of *E. coli* in stormwater biofilter media during the dry period, and different temperatures were tested as an operational factor. Simple linear regression was also employed to represent the dependency of *E. coli* die-off on temperature. Although this model did not incorporate any wet weather event, the attempts to include temperature as an operational factor improved the capability of this model for die-off simulation during dry periods.

According to the published literature, none of the available models could meet all the requirements listed in Section 2.6. Especially, the existing models hardly include all the governing processes and the operational factors. These suggest that none of the existing models for microbial removal could be applied in RTC strategy evaluation and scenario simulation. Therefore, a capable predictive model needed to be developed for RTC in stormwater biofilters.

2.6.2 Other available models for microbial transport prediction in porous media systems

Although there are only a few models have been developed for the faecal microorganisms removal in biofilters, some models developed for other filtration/infiltration applications (e.g., wastewater treatment and aquifer recharge) are also valuable as references. Therefore, although this kind of model might not be directly transferable because of the differences in inflow and system design, common approaches used in modelling microbial behaviour in porous media will also be discussed below.

The most widely modelling approach used for microbial transport prediction in porous media is the adsorption-dispersion equation (Foppen et al., 2007; Tufenkji, 2007). This equation models the movement of microbes through advection, dispersion, and retention-related processes such as adsorption, desorption and straining (Bradford et al., 2006; Gargiulo et al., 2008). In addition, the governing processes (i.e., adsorption, desorption, staining, and die-off), could be modelled using first-order kinetics equations (Crane and Moore, 1986). However, some studies aimed at small laboratory columns disregarded the die-off process due to shorter simulation periods (Bradford et al., 2006; Foppen et al., 2007).

All of the above processes can be summarised in three generic equations that simulate the microbial concentration in the liquid phase (Equation 2.1) and the solid phase (Equation 2.2 and Equation 2.3) within a porous medium at any given time, as shown below.

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} - v\frac{\partial C}{\partial x} - \left(k_{att}C - \frac{r_s}{q}k_{\det}S_1\right) - k_{str}j\,C - m_l C \qquad \text{Equation 2.1}$$

$$\frac{\partial S_1}{\partial t} = \frac{q}{r_s}k_{att}C - k_{\det}S_1 - m_s S_1 \qquad \text{Equation 2.2}$$

$$\frac{\partial S_2}{\partial t} = \frac{q}{r_s}k_{str}j\,C - m_s S_2 \qquad \text{Equation 2.3}$$

Where C is the microbial concentration in the liquid phase (cells/mL); S_1 is the microbial concentration in the solid phase due to kinetic attachment (cells/g); S_2 is the microbial concentration in the solid phase due to straining

(cells/g); t is the time (s); x is the downgradient distance from the inlet (cm); D is the hydrodynamic dispersion coefficient (cm^2/s); v is the pore water velocity (cm/s); ρ is the bulk density of the porous media (g/cm^3); n is the porosity (-); k_{att}, k_{det} and k_{str}, are the adsorption, desorption, and straining rates respectively (s^{-1}); μ_l and μ_s are the inactivation or die-off rates for liquid and solid phases respectively (s^{-1}); and φ is the fitting/reduction coefficient for straining (-).

2.6.3 Modelling of governing processes and impact of key factors

This section will discuss how to define the key parameters (e.g. k_{att}, k_{det}, k_{str}, μ_l, and μ_s) as well as the coefficients (e.g., φ) in Equations 2.1 ~ 2.3. The discussion will be structured according to the governing processes where these parameters and coefficients belong to.

(1) Adsorption

Adsorption, which was named as attachment in some literature, is considered as the major governing process for microbial removal. In most of the previous studies, adsorption was described with first-order attachment rate coefficient k_{att} (Hijnen et al., 2005; Tufenkji, 2007; Zhang et al., 2010).

When calculating k_{att}, a common approach employed in previous studies is the colloid filtration theory (Hijnen et al., 2005; Zhang et al., 2010):

$$k_{att} = \frac{3(1-q)v}{2d_c} h_0 a \qquad \text{Equation 2.4}$$

Where d_c is the average media grain size (cm); η_0 is the single media contact efficiency (-); α is the attachment coefficient (-), which was calculated from the bacterial breakthrough curves (Tufenkji and Elimelech, 2004):

$$a = -\frac{2}{3}\frac{d_c}{(1-q)Lh_0}\ln\frac{c}{c_0} \qquad \text{Equation 2.5}$$

Where L is the filter media packed length (L); c/c_0 is the normalized bacterial concentration in the column outlet at the initial stage of the particle breakthrough curve.

This approach allows reflect the characteristics of filter media, since d_c, η_0, and α are all depend on media properties. In addition, it represents the dependence between pore water velocity and attachment rate, which is, a higher pore water velocity could result in a higher attachment rate. However, this dependence was challenged by the findings of many previous studies, which indicated that higher microbial removal is achieved under lower infiltration rates due to enhanced adsorption was achieved at the lower infiltration rate (Bradford et al., 2006; Stevik et al., 2004; Tong and Johnson, 2006). Furthermore, Harter et al. (2000) observed a peculiar dependence between α and pore velocity: significantly decreases with decreasing pore velocity; however, it is not explained by the model colloid filtration theory. Therefore, the colloid filtration theory may have its limitations when describing the adsorption process.

(2) Desorption

Desorption was neglected in most of the previous studies (Bradford et al., 2006; Foppen et al., 2007; Zhang et al., 2010). The main reason is: some studies only simulated a single event and used extremely high inflow concentrations, as such desorption is less important compared to adsorption, especially if this single event occurred in a clean laboratory-scale column which is initially free of target microorganisms (Zhang et al., 2010). In addition, it was reported that the desorption rate is at least two to three orders of magnitude lower than the adsorption rate (Bradford et al., 2006). However, the presence of concentration tailing in the outflow breakthrough curves cannot be fully described and explained by employing the conventional colloid filtration theory when disregarding the desorption process. Furthermore, previous studies showed that the slow release of microorganisms that found in outflow breakthrough curves during subsequent dosing of microbial-free water is due to the desorption of microorganisms from porous media (Bales et al., 1991; Harter et al., 2000). Therefore, when developing a model for microbial removal in biofilter, it might be necessary to take desorption into consideration.

In the models that included desorption, this process was modelled as first-order kinetics as shown in Equation 2.3. In this method, desorption is highly depended on the adsorbed microbial concentration, and it is a one-site process.

However, some authors, like Johnson et al. (1995), modelled desorption as a two-site process where one fraction of reversible adsorbed microorganisms desorb at a faster rate within a very short time, whilst the other fraction desorbs at a relatively slow rate over a long period of time. Johnson et al. (1995) also reported that in their experiment, 90 % of the bacteria were desorbed within a very short time, and only the minority of slowly desorbed bacteria contributed to the tailing in the outflow breakthrough curve. According to these authors, desorption was modelled as:

$$\frac{M_t}{M_0} = Ae^{-k_{\det 1}t} + (1-A)e^{-k_{\det 2}t} \qquad \text{Equation 2.6}$$

Where M_t is the number of microorganisms remaining in solid phase due to reversible adsorption at time, t (cells), M_0 is the number of microorganisms reversibly adsorbed to the filter media initially (cells), A is the weighting factor (-) and k_{det1} and k_{det2} are the fast and slow desorption rate coefficients (s^{-1}).

However, considering the difficulty of defining the exact fractions of fast and slow desorption, the two-site desorption model has hardly been applied in the microbial removal in biofilters (Tong and Johnson, 2006; Zhang et al., 2010).

As discussed in Section 2.3.1, the faecal microbial attachment in stormwater biofilters would be mainly reversible because of the neutral pH and low ionic strengths in stormwater (Schijven and Hassanizadeh, 2000). Therefore, desorption is a significant process in stormwater biofilters and should be included in models related to microbial removal in stormwater biofilters.

(3) Straining

Like desorption, the significance of straining is often overlooked in previous studies (Hathaway et al., 2011; Zhang et al., 2010). However, Bradford et al. (2006) found that straining also plays a significant role in *E. coli* removal in the fine sand media. As shown in Equation 2.4, the authors also modelled straining as first order kinetics. In addition, the authors hypothesised that the sites available for straining decrease with depth, and the straining coefficient also dependent on media size. The model was described as Equation 2.7:

$$j = \left(1 - \frac{S_2}{S_2^{\max}}\right)\left(\frac{d_{50} + x}{d_{50}}\right)^{-b} \qquad \text{Equation 2.7}$$

Where S_2^{max} is the maximum microbial concentration in solid phase due to straining (cells/g), d_{50} is the average grain size (cm) and β is the dimensionless parameter that controls the spatial distribution of retained microorganisms (-).

However, considering the small value of S_2/S_2^{max}, the first term on the left side of Equation 2.7 is very close to 1 and was neglected by a later study conducted by the same research group (Gargiulo et al., 2008).

Results reported by Bradford et al. (2006) indicated a non-linear decrease in straining capacity with depth, but Foppen et al. (2007) found that straining occurs throughout the depth with a linearly decreasing trend. Chandrasena (2014) also reported that the majority of straining occurs in the top sediments in stormwater filters. These studies all proved that the effect of straining is highly dependent on the location in a biofilter.

Although some may argue that the straining coefficient also depends on time since the straining sites in a biofilter are limited, Haznedaroglu et al. (2009) found that filling of straining sites during a wet weather period was only observed under very high microbial concentration in inflow such as 10^8 cells/mL. Since such a high inflow microbial concentration has not been reported in stormwater, it is unlikely that straining will be limited due to the filling of available sites in stormwater biofilters during short wet weather periods with relatively low microbial concentrations in inflow.

Furthermore, according to Bradford et al. (2006), in sand based biofilter media, straining plays a significant role in removing microorganisms with a size larger than 0.425 μm. Since the sizes of all free phase protozoa, bacteria and some large viruses are larger than that, straining should be included as one of the major processes in any predictive model for stormwater biofilters.

(4) Die-off

Microbial die-off is an integral part of the faecal microbial behaviour, and typically modelled as first-order kinetics (Equation 2.8) (Crane and Moore, 1986; Yates and Yates, 1987). As discussed in Section 2.3.1, the die-off rate coefficient (μ) is highly dependent on various operational factors like temperature and moisture content. To simplify the model, most of the previous studies lumped the influence of operational conditions into a single μ value; however, some studies represented more complex functional dependencies to reveal the effect of each individual operational factor, and the overall die-off rate coefficient was be represented as a combination for each functional relationship (Equation 2.9) (Reddy et al., 1981; Zhang et al., 2012). According to Zhang *et al.* (2012), the impact of temperature is shown in Equation 2.10. In addition, based on the data reported by Boyd et al. (1969), Reddy et al. (1981) suggested an equation (Equation 2.11) to describe the impact of moisture content on the die-off rate:

$$N_t = N_0 e^{-\mu t} \qquad \text{Equation 2.8}$$

$$\mu = \mu_0 F_T F_M F_{pH} \qquad \text{Equation 2.9}$$

$$F_T = \theta^{(T - T_0)} \qquad \text{Equation 2.10}$$

$$F_M = \alpha + \beta(MC) \qquad \text{Equation 2.11}$$

Where N_t is the current microbial concentration in the media (cells/g or cells/mL), N_0 is the initial microbial concentration in the media (cells/g or cells/mL), μ_0 is the reference first order die-off rate coefficient at given reference conditions of the system of interest (e.g.: standard temperature, moisture content, pH) (s^{-1}), F_T, F_M and F_{pH} are the factor for temperature, moisture content and pH respectively (-), T is the temperature (°C), T_0 is the reference temperature (e.g. 20 °C) (°C), θ is the Temperature correction coefficient, MC is the moisture content (%), α are β the linear regression coefficients (-).

Since the contributions made by die-off during wet weather events are not as significant as other processes like adsorption, some studies neglected this process when modelling the wet weather events (Chandrasena et al., 2013; Zhang et al., 2010). However, die-off has been proved to be the major process

for microbial removal during the dry periods between wet weather events. Therefore, this process should be included in any predictive model that designed for microbial removal in stormwater biofilters.

2.6.4 Summary of existing models

Although several models have been developed to model the governing processes during wet weather events and/or dry periods in between, none of them could meet all the requirements listed at the beginning of Section 2.6. In other words, none of them is capable of being utilised as a predictive model for RTM/RTC of stormwater biofilters. However, it might be possible to incorporate the available methodologies for individual event/process/factor modelling, in order to develop a comprehensive predictive model for RTM/RTC for microbial removal in stormwater biofilters.

2.7 Conclusions of literature review and key knowledge gaps

The existing stormwater biofilters need to be optimised considering the high levels of microorganisms in urban stormwater runoff and the high requirements in Australia guidelines for stormwater harvesting and reuse as well other guidelines. In most previous studies, the optimisation of biofilters in microbial removal focused on the design perspective. Although some studies also revealed the importance of operational conditions, the optimisation of these operational conditions has hardly been applied due to the difficulty of implementation. To solve this problem, RTC technology is a potentially effective tool. Although RTC has been applied in some other domains in the environmental field, currently no publication has been found to discuss the application of RTC in stormwater biofilters. Therefore, a framework for RTC of stormwater biofilters should be developed.

According to the characteristic of biofilters and faecal microorganisms, the framework of RTC in stormwater biofilters may include developing predictive models for microbial removal prediction, developing RTC strategies, testing

RTC strategies through practical implementation, and use the developed model to systematically evaluate RTC strategies.

The key knowledge gaps to for the development of the framework of RTC in stormwater biofilters include:

(1) Lack of a predictive model of microbial removal in biofilters to serve as the fundamental part of RTC for strategy evaluation and selection, which should include the governing processes and key factors, and represent both wet weather events and dry periods;
(2) Lack of RTC data for model development;
(3) No RTC strategy for stormwater biofilters has ever been developed;
(4) The outcomes of adopting different RTC strategies in various systems (i.e., with various sizes, locations, and under different weather conditions) are unknown;
(5) Lack of knowledge about if the RTC strategies design for the optimisation of faecal microbial removal would worsen the treatment of other pollutants;
(6) How to balance the competing needs for stormwater harvesting and reuse (e.g., high water volume and good water quality) that may exist in RTC implementation needs to be studied.

2.8 Research questions and hypotheses

To address the research gaps listed in Section 2.7, three high-level research questions and hypotheses are formulated.

Research Question 1: What components need to be included and what equations could be adopted in a model that developed for the prediction of faecal microbial removal in stormwater biofilters?

- It is hypothesised that the governing processes for faecal microbial removal in biofilters, adsorption, desorption, straining, and survival/die-off, need to be included in the model;
- It is hypothesised that the key operational factors for faecal microbial removal in biofilters, such as temperature and moisture content, need to be included in the model;

- It is hypothesised that advection-dispersion equations can be employed to simulate the transport and fate of faecal microbes within biofilters.

Research Question 2: What are the criteria and what information and facilities could be adopted in developing an effective RTC strategy for stormwater harvesting and reuse?

- It is hypothesised that the criteria for an effective RTC strategy include be capable of providing large volume and good quality of water for harvesting, could well protect the environment, be adjustable to various design features and operational conditions, be easy to implement, and need low cost;
- It is hypothesised that the forecast and real-time information of weather (e.g. rainfall forecast, rainfall duration, and number of dry days), inflow characteristics (e.g. inflow rate and concentration), and operational conditions of biofilters (e.g. temperature, moisture content, the depth of submerged zone) could be adopted to develop an effective RTC strategy;
- It is hypothesised that different types of sensors, valves in pipes, timers, and flowmeters could be adopted to develop an effective RTC strategy.

Research Question 3: What benefits could RTC provide, and how select the optimum RTC strategy in a certain case to balance the competing needs that may exist in stormwater harvesting and reuse?

- It is hypothesised that different RTC strategies could provide different benefits, e.g., some could provide a large volume of water for harvesting, some could provide extremely high water quality for harvesting, while some could only provide benefits for harvesting but also protect the environment well.
- It is hypothesised that even a same RTC strategy could provide different benefits when being applied in different systems with various design features and inflow characteristics (e.g., inflow volume and concentration);
- It is hypothesised that using the modelling tool to explore a large amount of RTC strategies and test numerous scenarios, with the consideration

of treatment requirements (e.g., for different end uses), could help to figure out the optimum RTC strategy and balance the competing needs that may exist in stormwater harvesting and reuse.

2.9 References

Alexander M. Why microbial predators and parasites do not eliminate their prey and hosts. Annual Review of Microbiology 1981; 35: 113-133.

Arnone R. D., Walling J. P. Waterborne pathogens in urban watersheds. Journal of Water and Health 2007; 5: 149-162.

Bales R. C., Hinkle S. R., Kroeger T. W., Stocking K., Gerba C. P. Bacteriophage adsorption during transport through porous media: Chemical perturbations and reversibility. Environmental Science & Technology 1991; 25: 2088-2095.

Berndtsson J. C. Green roof performance towards management of runoff water quantity and quality: A review. Ecological Engineering 2010; 36: 351-360.

Bitton G., Gerba C. P. Microbial Pollutants: Their Survival and Transport Pattern to Groundwater. Groundwater Pollution Microbiology. John Wiley and Sons, New York, USA, 1984.

Boyd J. W., Yoshida T., Vereen L. E., Cada R. L., Morrison S. M. Bacterial Response to the Soil Environment. Colorado Water Resources Research Institute, Colorado State University, Fort Collins, Colorado, 1969.

Bradford S. A., Simunek J., Walker S. L. Transport and straining of *E. coli* O157:H7 in saturated porous media. Water Resources Research 2006; 42: W12S12.

Bratieres K., Fletcher T. D., Deletic A., Zinger Y. Nutrient and sediment removal by stormwater biofilters: A large-scale design optimisation study. Water Research 2008; 42: 3930-3940.

Bright T., Hathaway J., Hunt W., III, de los Reyes F., III, Burchell M., II. Impact of storm-water runoff on clogging and fecal bacteria reduction in sand columns. Journal of Environmental Engineering 2010; 136: 1435-1441.

Brookes J., Hipsey M., Burch M. D., Linden L. G., Ferguson C., Antenucci J. Relative value of surrogate indicators for detecting pathogens in lakes and reservoirs. Environmental Science & Technology 2005; 39: 8614-8621.

Brookes J. D., Antenucci J., Hipsey M., Burch M. D., Ashbolt N. J., Ferguson C. Fate and transport of pathogens in lakes and reservoirs. Environment International 2004; 30: 741-759.

Burton G. A., Gunnison D., Lanza G. R. Survival of pathogenic bacteria in various freshwater sediments. Applied and Environmental Microbiology 1987; 53: 633-638.

Carleton J. N., Grizzard T. J., Godrej A. N., Post H. E., Lampe L., Kenel P. P. Performance of constructed wetlands in treating urban stormwater runoff. Water Environment Research 2000; 72: 295-304.

Chandrasena G. I. Faecal Microorganism Removal in Stormwater Biofilters. Department of Civil Engineering. Doctor of Philosophy. Monash University, 2014.

Chandrasena G. I., Deletic A., A. L., Henry R., McCarthy D. T. Stormwater biofilters as barriers against *Campylobacter jejuni*, *Cryptosporidium* oocysts and adenoviruses; results from a laboratory trial. Water 2017; 9: 949.

Chandrasena G. I., Deletic A., Ellerton J., McCarthy D. T. Evaluating *Escherichia coli* removal performance in stormwater biofilters: a laboratory-scale study. Water Science & Technology 2012; 66: 1132-1138.

Chandrasena G. I., Deletic A., McCarthy D. T. Evaluating *Escherichia coli* removal performance in stormwater biofilters: a preliminary modelling approach. Water Science & Technology 2013; 67: 2467-75.

Chandrasena G. I., Deletic A., McCarthy D. T. Survival of *Escherichia coli* in stormwater biofilters. Environmental Science and Pollution Research 2014a; 21: 391-401.

Chandrasena G. I., Deletic A., McCarthy D. T. Biofiltration for stormwater harvesting: Comparison of *Campylobacter spp.* and *Escherichia coli* removal under normal and challenging operational conditions. Journal of Hydrology 2016; 537: 248-259.

Chandrasena G. I., Pham T., Payne E. G., Deletic A., McCarthy D. T. *E. coli* removal in laboratory scale stormwater biofilters: Influence of vegetation and submerged zone. Journal of Hydrology 2014b; 519: 814-822.

Chang L.-C., Chang F.-J. Intelligent control for modelling of real-time reservoir operation. Hydrological Processes 2001; 15: 1621-1634.

Cizek A. R., Characklis G. W., Krometis L. A., Hayes J. A., Simmons III O. D., Di Lonardo S., et al. Comparing the partitioning behavior of Giardia and Cryptosporidium with that of indicator organisms in stormwater runoff. Water Research 2008; 42: 4421-4438.

Corominas L., Sin G., Puig S., Traore A., Balaguer M., Colprim J., et al. Model-based evaluation of an on-line control strategy for SBRs based on OUR and ORP measurements. Water Science & Technology 2006; 53: 161.

Coustumer S. L., Fletcher T. D., Deletic A., Barraud S., Poelsm P. The influence of design parameters on clogging of stormwater biofilters: A large-scale column study. Water Research 2012; 46: 6743–6752.

Crane S. R., Moore J. A. Modeling Enteric Bacterial Die-off - A Review. Water, Air, and Soil Pollution 1986; 27: 411-439.

CWSC. Project 1: Sustainable Technologies. Centre for water sensitive cities (CWSC), Australia, 2010.

Czemiel Berndtsson J. Green roof performance towards management of runoff water quantity and quality: A review. Ecological Engineering 2010; 36: 351-360.

Davies C. M., Bavor H. J. The fate of stormwater associated bacteria in constructed wetland and water pollution control pond systems. Journal of Applied Microbiology 2000; 89: 349-360.

Davies C. M., Yousefi Z., Bavor H. J. Occurrence of coliphages in urban stormwater and their fate in stormwater management systems. Letters in Applied Microbiology 2003; 37: 299-303.

Díaz F. J., O'Geen A. T., Dahlgren R. A. Efficacy of Constructed Wetlands for Removal of Bacterial Contamination From Agricultural Return Flows. Agricultural Water Management 2010; 97: 1813-1821.

Duncan H. P. Urban Stormwater Quality: A Statistical Overview. Melbourne, Australia: Cooperative Research Centre for Catchment Hydrology, 1999.

Ecowise. Characterisation of human health risk derived from stormwater. Ecowise Environmental PTY Ltd., 2010.

Edberg S. C., Rice E. W., Karlin R. J., Allen M. J. Escherichia coli: The best biological drinking water indicator for public health protection. Journal of Applied Microbiology Symposium Supplement 2000; 88: 106s-116s.

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

Feng W., Hatt B. E., McCarthy D. T., Flecther T. D., Deletic A. Biofilters for stormwater harvesting: Understanding the treatment performance of key metals that pose a risk for water use. Environmental Science & Technology 2012; 46: 5100-5108.

Ferguson C., De Roda Husman A. M., Altavilla N., Deere D., Ashbolt N. Fate and transport of surface water pathogens in watersheds. Critical Reviews in Environmental Science and Technology 2003; 33: 299-361.

Fletcher T. D., Mitchell V. G., Deletic A., Ladson A. Is stormwater harvesting beneficial to urban waterway flow? Water Science & Technology 2007; 55: 265-272.

Foppen J. W., van Herwerden M., Schijven J. Measuring and modelling straining of *Escherichia coli* in saturated porous media. Journal of Contaminant Hydrology 2007; 93: 236-54.

Fowdar H. S., Hatt B. E., Breen P., Cook P. L. M., Deletic A. Designing living walls for greywater treatment. Water Research 2017; 110: 218-232.

Franchi A., O'Melia C. R. Effects of natural organic matter and solution chemistry on the deposition and reentrainment of colloids in porous media. Environmental Science & Technology 2003; 37: 1122-1129.

Gaborit E., Muschalla D., Vallet B., Vanrolleghem P. A., Anctil F. Improving the performance of stormwater detention basins by real-time control using rainfall forecasts. Urban Water Journal 2013; 10: 230-246.

Gargiulo G., Bradford S., Šimůnek J., Ustohal P., Vereecken H., Klumpp E. Bacteria transport and deposition under unsaturated conditions: the role of the matrix grain size and the bacteria surface protein. Journal of Contaminant Hydrology 2007; 92: 255-273.

Gargiulo G., Bradford S. A., Simunek J., Ustohal P., Vereecken H., Klumpp. E. Bacteria Transport and Deposition under Unsaturated Flow Conditions: The Role of Water Content and Bacteria Surface Hydrophobicity. Vadose Zone Journal 2008; 7: 406-419.

Garzio-Hadzick A., Shelton D. R., Hill R. L., Pachepsky Y. A., Guber A. K., Rowland R. Survival of manure-borne E. coli in streambed sediment: effects of temperature and sediment properties. Water Research 2010; 44: 2753-2762.

Han H., Qiao J. Nonlinear Model-Predictive Control for Industrial Processes: An Application to Wastewater Treatment Process. IEEE TRANSACTIONS ON INDUSTRIAL ELECTRONICS 2014; 61: 1970-1980.

Harter T., Wagner S., Atwill E. R. Colloid transport and filtration of Cryptosporidium parvum in sandy soils and aquifer sediments. Environmental Science & Technology 2000; 34: 62-70.

Hathaway J., Hhunt W., Graves A., Wright J. Field Evaluation of Bioretention Indicator Bacteria Sequestration in Wilmington, North Carolina. Journal of Environmental Engineering 2011; 137: 1103-1113.

Hathaway J., Hunt W. Indicator Bacteria Removal by Stormwater BMPs in Coastal North Carolina. World Environmental and Water Resources Congress: Challenges of Change, Providence, Rhode Island, United States, 2010, pp. 3218-3225.

Hathaway J. M., Hunt W. F., Jadlocki S. Indicator bacteria removal in storm-water best management practices in charlotte, north carolina. Journal of Environmental Engineering 2009; 135: 1275-1285.

Hatt B. E., Deletic A., Fletcher T. D. Integrated Stormwater Treatment and Re-use Systems - Inventory of Australian practice. Cooperative Research Centre for Catchment Hydrology, Melbourne, Australia, 2004.

Hatt B. E., Deletic A., Fletcher T. D. Integrated treatment and recycling of stormwater: a review of Australian practice. J Environ Manage 2006; 79: 102-13.

Hatt B. E., Fletcher T. D., Deletic A. Hydraulic and pollutant removal performance of stormwater filters under variable wetting and drying regimes. Water Science & Technology 2007; 56: 11-19.

Haznedaroglu B. Z., Kim H. N., Bradford S. A., Walker S. L. Relative transport behavior of Escherichia coli O157:H7 and Salmonella enterica serovar pullorum in packed bed column systems: Influence of solution chemistry and cell concentration. Environmental Science & Technology 2009; 43: 1838-1844.

Hijnen W. A. M., Brouwer-hanzens A. J., Charles K. J., Medema G. J. Transport of MS2 phage, Escherichia coli Clostridium perfringens, Cryptosporidium parvum, and Giardia intestinalis in a gravel and a sandy soil. Environmental Science and Technology 2005; 39: 7860-7868.

Hipsey M. R., Antenucci J. P., Brookes J. D. A generic, process-based model of microbial pollution in aquatic systems. Water Resources Research 2008; 44.

Horan N. J. Faecal indicator organisms. The Handbook of Water and Wastewater Microbiology. Academic Press, United Kingdom, 2003.

Hsu N.-S., Huang C.-L., Wei C.-C. Multi-phase intelligent decision model for reservoir real-time flood control during typhoons. Journal of Hydrology 2015; 522: 11-34.

Johnson W. P., Zhang P., Gradner P. M., Fuller M. E., Deflaun M. F. Modeling bacterial detachment during transport through porous media as a residence-time-dependent process. Applied and Environmental Microbiology 1995; 67: 4908-4913.

Jones M. P., Hunt W. F. Bioretention impact on runoff temperature in trout sensitive waters. Journal of Environmental Engineering 2009; 135: 577-585.

Kadlec R. H., Wallace S. Treatment wetlands (Second edition): CRC Press, 2008.

Kandra H. S., McCarthy D., Fletcher T. D., Deletic A. Assessment of clogging phenomena in granular filter media used for stormwater treatment. Journal of Hydrology 2014; 512: 518-527.

Kerkez B., Gruden C., Lewis M., Montestruque L., Quigley M., Wong B., et al. Smarter Stormwater Systems. Environmental Science & Technology 2016; 50: 7267-7273.

Krometis L.-A. H., Drummey P. N., Characklis G. W., Sobsey M. D. Impact of microbial partitioning on wet retention pond effectiveness. Journal of Environmental Engineering 2009; 135: 758-767.

Langeveld J. G., Benedetti L., de Klein J. J. M., Nopens I., Amerlinck Y., van Nieuwenhuijzen A., et al. Impact-based integrated real-time control for improvement of the Dommel River water quality. Urban Water Journal 2013; 10: 312-329.

Le Coustumer S., Fletcher T. D., Deletic A., Barraud S., Poelsma P. The influence of design parameters on clogging of stormwater biofilters: a large-scale column study. Water Research 2012; 46: 6743-52.

Leon A. S., Kanashiro E. A., Valverde R., Sridhar V. Dynamic Framework for Intelligent Control of River Flooding: Case Study. Journal of Water Resources Planning and Management 2014; 140: 258-268.

Li Y., McCarthy D. T., Deletic A. *Escherichia coli* removal in copper-zeolite-integrated stormwater biofilters: Effect of vegetation, operational time, intermittent drying weather. Ecological Engineering 2016; 90: 234-243.

Li Y. L., Deletic A., Alcazar L., Bratieres K., Fletcher T. D., McCarthy D. T. Removal of Clostridium perfringens, Escherichia coli and F-RNA coliphages by stormwater biofilters. Ecological Engineering 2012; 49: 137-145.

Lim C. H., Flint K. P. The effect of nutrients on the survival of Escherichia coli in lake water. Journal of Applied Bacteriology 1989; 66: 559-569.

Lund N. S. V., Falk A. K. V., Borup M., Madsen H., Mikkelsen P. S. Model predictive control of urban drainage systems: A review and perspective towards smart real-time water management. Critical Reviews in Environmental Science and Technology 2018; 48: 279-339.

Makepeace D. K., Smith D. W., Stanley S. J. Urban stormwater quality: Summary of contaminant data. Critical Reviews in Environmental Science and Technology 1995; 25: 93-139.

Malaviya P., Singh A. Constructed Wetlands for Management of Urban Stormwater Runoff. Critical Reviews in Environmental Science and Technology 2012; 42: 2153-2214.

Mallin M. A., Ensign S. H., Wheeler T. L., Mayes D. B. Pollutant removal efficacy of three wet detention ponds. Journal of Environmental Quality 2002; 31: 654-660.

Meneses E. J., Gaussens M., Jakobsen C., Mikkelsen P. S., Grum M., Vezzaro L. Coordinating Rule-Based and System-Wide Model Predictive Control Strategies to Reduce Storage Expansion of Combined Urban Drainage Systems: The Case Study of Lundtofte, Denmark. Water 2018; 10: 15.

Meng Z., Chandrasena G., Henry R., Deletica A., Kolotelo P., McCarthy D. Stormwater constructed wetlands: A source or a sink of *Campylobacter* spp. Water Research 2018; 131: 218-227.

Mukerji K. G., Manoharachary C., Singh J. Microbial Activity in the Rhizosphere. Berlin: Berlin Springer, 2006.

Mullapudi A., Bartos M., Wong B., Kerkez B. Shaping Streamflow Using a Real-Time Stormwater Control Network. Sensors 2018; 18: 2259.

Murphy H. M., Meng Z., Henry R., Deletic A., McCarthy D. T. Current Stormwater Harvesting Guidelines Are Inadequate for Mitigating Risk from *Campylobacter* During Nonpotable Reuse Activities. Environmental science & technology 2017; 51: 12498-12507.

NHMRC. Australian Drinking Water Guidelines. Australian Government, National Health and Medical Research Council, 2004.

NHMRC. Guidelines for Managing Risks in Recreational Water. Australian Government, National Health and Medical Research Council, Canberra, Australia, 2008.

NHMRC. Australian Guidelines for Water Recycling (Phase 2). Stormwater Harvesting and Reuse. Australian Government, Natural Resource Management Ministerial Council, Environment Protection and Heritage Council, National Health and Medical Research Council, Canberra, Australia, 2009.

Oberndorfer E., Lundholm J., Bass B., Coffman R. R., Doshi H., Dunnett N., et al. Green roofs as urban ecosystems: Ecological structures, functions, and services. Bioscience 2007; 57: 823-833.

Oliveri V. P., Kruse C. W., Kawata K. Microorganisms in Urban Stromwater (EPA-600/2-77-087). US EPA, 1977.

Pachepsky Y. A., Shelton D. R. Escherichia Coliand Fecal Coliforms in Freshwater and Estuarine Sediments. Critical Reviews in Environmental Science and Technology 2011; 41: 1067-1110.

Payne E. G. I., Pham T., Cook P. L. M., Fletcher T. D., Hatt B. E., Deletic A. Biofilter design for effective nitrogen removal from stormwater – influence of plant species, inflow hydrology and use of a saturated zone. Water Science & Technology 2014; 69: 1312-1319.

Perdek J. M., Arnone R. D., Stinson M. K., Tuccillo M. E. Managing Urban Watershed Pathogen Contamination. U.S. Environmental Protection Agency, Cincinnati, Ohio, 2003.

Reddy K. R., Khaleel R., Overcash M. R. Behavior and transport of microbial pathogens and indicator organisms in soils treated with organic wastes. Journal of Environmental Quality 1981; 10: 255-266.

Redman J. A., Walker S. L., Elimelech M. Bacterial Adhesion and Transport in Porous Media: Role of the Secondary Energy Minimum. Environmental Science & Technology 2004; 38: 1777-1785.

Rusciano G. M., Obropta C. C. Bioretention column study: Fecal coliform and total suspended solids reductions. Transactions of the ASABE 2007; 50: 1261-1269.

Schijven J. F., Hassanizadeh S. M. Removal of viruses by soil passage: Overview of modeling, processes, and parameters. Critical Reviews in Environmental Science and Technology 2000; 30: 49-127.

Schilling W., Andersson B., Nyberg U., Aspegren H., Rauch W., Harremoës P. Real time control of wastewater systems. Journal of Hydraulic Research 1996; 34: 785-797.

Schultz N., Meagher D., Cabibel B. Milwaukee's Experience in Collection System Controls. Proceedings of Water Environment Federation Specialty Conference 2001, 2001.

Schütze M., Butler D., Beck B. M. Modelling, Simulation and Control of Urban Wastewater Systems: Springer Science & Business Media, 2002.

Schütze M., Campisano A., Colas H., Schilling W., Vanrolleghem P. A. Real time control of urban wastewater systems—where do we stand today? Journal of Hydrology 2004; 299: 335-348.

Stenstrom T. A., Carlander A. Occurrence and die-off of indicator organisms in the sediment in two constructed wetlands. Water Science & Technology 2001; 44: 223-230.

Stevik T. K., Aa K., Ausland G., Hanssen J. F. Retention and removal of pathogenic bacteria in wastewater percolating through porous media: a review. Water Research 2004; 38: 1355-1367.

Stinson M. Benefits of Sewerage System Real-Time Control. World Water and Environmental Resources Congress 2005, Anchorage, Alaska, United States, 2005, pp. 1-11.

Tian X., van Overloop P.-J., Negenborn R. R., van de Giesen N. Operational flood control of a low-lying delta system using large time step Model Predictive Control. Advances in Water Resources 2015; 75: 1-13.

Tong M., Johnson W. P. Excess Colloid Retention in Porous Media as a Function of Colloid Size, Fluid Velocity, and Grain Angularity. Environmental Science & Technology 2006; 40: 7725-7731.

Tufenkji N. Modeling microbial transport in porous media: Traditional approaches and recent developments. Advances in Water Resources 2007; 30: 1455-1469.

Tufenkji N., Elimelech M. Correlation equation for predicting single-collector efficiency in physicochemical filtration in saturated porous media. Environmental Science & Technology 2004; 38: 529-536.

Van Mechelen C., Dutoit T., Hermy M. Mediterranean open habitat vegetation offers great potential for extensive green roof design. Landscape and Urban Planning 2014; 121: 81-91.

Vandenhove H., Merckx R., Wilmots H., Vlassak K. Survival of Pseudomonas-Fluorescens Inocula of Different Physiological Stages in Soil. Soil Biology & Biochemistry 1991; 23: 1133-1142.

Wadzuk B. M., Rea M., Woodruff G., Flynn K., Traver R. G. Water-quality performance of a constructed stormwater wetland for all flow conditions. Journal of the American Water Resources Association 2010; 46: 385-394.

WERF. Development of a Protocol for Risk Assesment of Microorganisms in Seperate Stormwater Systems. Water Environment Research Foundation, 2007.

Willey J. M., Sherwood L. M., Woolverton C. J. Prescott's Microbiology (8th Edition). New York, USA: McGraw-Hill, 2011.

Wong T. H. F. Australian runoff quality: a guide to water sensitive urban design. Crows Nest, N.S.W., Australia: Engineers Australia, 2006.

Yang Q., Peng Y., Liu X., Zeng W., Mino T., Satoh H. Nitrogen Removal via Nitrite from Municipal Wastewater at Low Temperatures using Real-Time Control to Optimize Nitrifying Communities. Environmental Science & Technology 2007; 41: 8159-8164.

Yates M. V., Yates S. R. Modeling microbial fate in the subsurface environment. 1987; 17: 307-344.

Zhang L., Seagren E. A., Davis A. P., Karns J. S. The Capture and Destruction of *Escherichia coli* from Simulated Urban Runoff Using Conventional Bioretention Media and Iron Oxide-coated Sand. Water Environment Research 2010; 82: 701-714.

Zhang L., Seagren E. A., Davis A. P., Karns J. S. Long-term sustainability of escherichia coli removal in conventional bioretention media. Journal of Environmental Engineering 2011; 137: 669-677.

Zhang L., Seagren E. A., Davis A. P., Karns J. S. Effects of temperature on bacterial transport and destruction in bioretention media: Field and laboratory evaluations. Water Environment Research 2012; 84: 485-496.

Zinger Y., Blecken G.-T., Fletcher T. D., Viklander M., Deletić A. Optimising nitrogen removal in existing stormwater biofilters: Benefits and tradeoffs of a retrofitted saturated zone. Ecological Engineering 2013; 51: 75-82.

Zinger Y., Deletic A., Fletcher T. D., Breen P., Wong T. A Dual-mode Biofilter System: Case study in Kfar Sava, Israel. 12th International Conference on Urban Drainage, Porto Alegre, Brazil, 2011.

Chapter 3.

Development and testing of stormwater biofilter treatment model for faecal microorganisms

3.1 Introduction

To achieve real time control (RTC), there are two types of strategies: off-line strategy and on-line strategy (Schütze et al., 2002). In off-line strategy, operational objectives are specified prior to the actual control process, set-points are normally fixed; while in on-line strategy, operational objectives are defined as a mathematical function, and set-points are variable and determined during the control process (Schütze et al., 2002). For RTC in stormwater biofilters, off-line strategies are preferred compared to on-line strategies, and the reasons include: (1) stormwater biofilters are rather simple low-tech systems, and (2) stormwater biofilter with RTC are required to react rapidly during rainfall events with short duration, and an off-line strategy normally needs less time to trigger control than an on-line strategy by avoiding to re-determine set-points (Lund et al., 2018).

To explore the potential off-line strategies for stormwater biofilters, using laboratory experiments and field tests to test each strategy is impractical, due to the large number of strategy, the high cost of experimental tests, and the long duration of each test. Another option for the evaluation of RTC strategies is modelling, as it is a more cost-effective tool that requires much less time and resources. Therefore, a predictive model for RTC serves as a fundamental part to achieve RTC in stormwater biofilters.

A capable predictive model is expected to include the governing processes (e.g., adsorption, desorption, and die-off) and operational factors (e.g., temperature) of microbial removal in biofilters, as without them, the microbial behaviours could not be sufficiently represented. In addition, the model should be able to simulate different operational conditions (e.g., infiltration rate, inflow volume, and inflow concentration) and reflect how these conditions would impact the transport and fate of microbes. The reason is, the function of RTC is to optimise these operational conditions in stormwater biofilters; if the model is not sensitive to these operational conditions, the effects of RTC could not be well predicted. Moreover, considering the large amount of potential strategies and scenarios, the difficulty in data collection, and the short during of some rainfall events, the

model that could generate accurate predictions with minimum data requirements and simulation time is favoured.

However, there is no available model that could meet all the above requirements. The previously developed models either focused only on one single wet event or dry period rather than continuous simulation of wet-dry-wet weather patterns (e.g., the models developed by Zhang et al. (2010) and Zhang et al. (2012)), or did not include all the governing processes and key factors (e.g., the one develop by Chandrasena et al. (2013)). Therefore, a model should be developed to fulfil these requirements.

It is also noted that, currently there are no RTC data available for stormwater biofilters, while a large amount of data have been collected for typical biofilters without RTC (non-RTC). Therefore, the first step of model development for RTC is to develop a model for typical biofilters without RTC, and this non-RTC model may need to be modified for RTC simulation later when RTC data are available.

As such, the objective of this study is using currently available data to develop a model (for non-RTC biofilters) to simulate the microbial removal in biofilters. This model should also have the potential to be applied for RTC simulation after modification. The research questions include:

- What components should be included in the model to adequately simulate the faecal microbial removal in stormwater biofilters?
- What equations could be utilised to model the transport and fate of faecal microbes within biofilters?
- How will the model perform when being applied in biofilters with various design features (e.g., different plan types and filter media types)?

Corresponding to the research questions, three hypotheses were made:

- faecal microbial removal in stormwater biofilters can be adequately simulated by modelling the governing processes (e.g., adsorption, desorption and die-off) that associated with key operational factors (e.g., temperature);

- the advection-dispersion equations and first-order kinetics equations can be employed to simulate the transport and fate of microbes in biofilters;
- the model is expected to perform generally consistent when being applied in different biofilters configurations; however, the differences in some design features (e.g., plant root size) may introduce extra uncertainties in prediction.

This chapter presents the processes of model development and testing based on a set of laboratory-scale column experiments. The results of sensitivity analysis and model validation are also included in this chapter to provide a comprehensive evaluation of the presented model.

The chapter is reproduced from an article published in *Science of the Total Environment* and presented in the original format of the journal. The citation is:

> **Pengfei Shen**, Ana Deletic, Christian Urich, Gayani I. Chandrasena, David T. McCarthy (2018). Stormwater biofilter treatment model for faecal microorganisms. Science of the Total Environment, 630, 992 – 1002.

3.2 Stormwater biofilter treatment model for faecal microorganisms

Science of the Total Environment 630 (2018) 992–1002

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Stormwater biofilter treatment model for faecal microorganisms

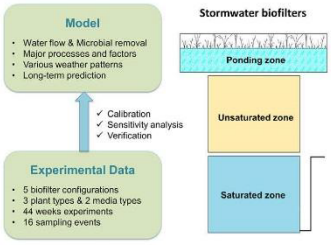
Pengfei Shen [a,b], Ana Deletic [a,c], Christian Urich [b], Gayani I. Chandrasena [a,b], David T. McCarthy [a,b,*]

[a] Environmental and Public Health Microbiology Laboratory (EPHM Lab), Department of Civil Engineering, Monash University, Clayton, VIC 3800, Melbourne, Australia
[b] Department of Civil Engineering, Monash University, Clayton, VIC 3800, Melbourne, Australia
[c] School of Civil and Environmental Engineering, University of New South Wales, Sydney, NSW 2052, Australia

HIGHLIGHTS

- Key processes and factors for microbial removal in biofilters were modelled.
- The prediction fitted well with the observation on five biofilter configurations.
- Microbial behavior under various operational conditions could be well simulated.
- Sensitivity analysis and validation were conducted for each biofilter configuration.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 20 November 2017
Received in revised form 16 February 2018
Accepted 16 February 2018
Available online xxxx

Editor: D. Barcelo

Keywords:
Stormwater
Biofilter
Modelling
Faecal microorganisms
Microbial removal
Escherichia coli

ABSTRACT

This paper presents a new model to simulate long-term microbial removal in stormwater biofilters. The water flow module uses a 'three-bucket' approach to describe the flow processes in biofilters, while the microbial quality module employs the one-dimensional advection-dispersion equation to represent microbial transport and fate under different design and operational conditions. Three governing processes for microbial removal, adsorption, desorption and die-off, are included; temperature is also incorporated as a key factor for die-off. The model was tested using long term monitoring data collected from laboratory columns in which five different biofilter configurations were studied over a period of 44 weeks. A multi-objective calibration with the balance of instantaneous ponding levels and event outflow volumes was implemented on the water flow module, and the Nash-Sutcliffe Efficiency (E) values ranged from 0.82 to 0.95. The microbial quality module was tested using the effluent *Escherichia coli* concentration data, and the E values obtained for different configurations were between 0.46 and 0.68. The optimized parameter values agreed with those presented in literature. However, sensitivity analyses suggested that the model's prediction was not sensitive to all parameters, the explanation for which was hypothesized to be data paucity rather than model structural uncertainties. Model validation was also conducted by splitting the data into calibration and validation datasets. The results further reinforced the needed for more data for model calibration.

* Corresponding author at: Environmental and Public Health Microbiology Laboratory (EPHM Lab), Department of Civil Engineering, Monash University, Clayton, VIC 3800, Melbourne, Australia.
E-mail addresses: [redacted] (P. Shen), [redacted] (D.T. McCarthy).

1. Introduction

Faecal microorganisms contained in stormwater runoff have been identified as one of the major pollutants impacting and degrading waterways around the globe (Burton and Pitt, 2002; Ferguson et al.,

https://doi.org/10.1016/j.scitotenv.2018.02.193
0048-9697/

2003). They are also the main pollutant that impedes stormwater harvesting due to the risks they pose when they come into contact with humans (Fletcher et al., 2008).

To reduce the harm caused by stormwater pollutants, biofilters (also known as rain gardens or bioretention systems) are widely applied as a part of Water Sensitive Urban Design (FAWB, 2009). While biofilters' sediment and nutrient removal capabilities are well documented, their promising ability to remove faecal microorganisms from stormwater has only recently been reported (Chandrasena et al., 2016; Chandrasena et al., 2014; Hathaway et al., 2011; Li et al., 2012). Previous studies indicated that the governing processes for the removal of faecal microbes in biofilters include adsorption, desorption and die-off (Bradford et al., 2006; Hathaway et al., 2011; Zhang et al., 2011).

Adsorption could be explained by the double layer theory, which describes the repulsive and attractive forces between microbes and media (Stevik et al., 2004). Since adsorption is reversible within the neutral pH range (Schijven and Hassanizadeh, 2000), which is also the pH range of stormwater (Duncan, 1999), desorption also occurs in biofilters. Adsorption and desorption govern the exchange of the mass of microbes between the liquid phase and solid phase in biofilters. These two processes are governed by different design and operational conditions such as media type, plant type, and flow conditions. Die-off controls the survival of microbes that are trapped by the soil and vegetation, and it is the dominant process during dry weather periods between wet weather events (Chandrasena et al., 2013). The effects of die-off are influenced by various abiotic (e.g. temperature and moisture content) (Ferguson et al., 2003; Schijven and Hassanizadeh, 2000; Zhang et al., 2011) and biotic conditions (e.g. predation and competition) (Flint, 1987; Zhang et al., 2010). Among these conditions, temperature is one of the most significant factors (Chandrasena et al., 2014).

Modelling is an important tool for better understanding the mechanisms of the removal of faecal microbes in biofilters, and for optimizing their design and operation. However, models for microbial removal in stormwater biofilters are very rare and not fully developed. Zhang et al. (2010) utilized a one-dimensional advection-dispersion equation to model the transport of *E. coli* in bioretention media during six hours of continuously simulated run-off conditions. Unfortunately, they only considered the processes of adsorption and die-off during a single rainfall event, without describing the influence of any operational factors or simulating dry periods, even though these are known to govern microbial removal in stormwater biofilters (Chandrasena et al., 2014; Chandrasena et al., 2014). Zhang et al. (2012) used the first order kinetic model to simulate the die-off process in stormwater biofilter media during dry periods. Although different temperatures were tested as an operational factor, no wet weather events were incorporated. As a result, governing processes for microbial removal that mainly take place during wet weather events, such as adsorption and desorption, were neglected. Therefore, this model was not able to simulate processes that occur during the period between two storm events that are important for overall system performance. Chandrasena et al. (2013) developed a model to simulate the outflow concentrations continuously, but this model is incapable of revealing the true transport of microorganisms throughout the biofilters as only a conceptual transport model was used. In addition, the impact of some operational factors such as temperature was not included. In summary, there is no available model that can continuously predict microbial removal in stormwater biofilters over extended periods.

Randelovic et al. (2016) presented a biofilter model for the treatment of micropollutants: a three-bucket approach was used to simulate biofilters; water flow and pollutant transport were modelled separately; the adsorption-dispersion equation was utilized to simulate pollutant transport. The model is capable of predicting the removal of different micro-pollutants in field-scale biofilters. However, the model was established for micropollutants removal and is unlikely to be suitable for microbial removal; furthermore, the model could neither simulate the differences in evapotranspiration rate between different plants, nor reflect the plant roots' capability of adsorbing water from different parts of a biofilter.

The main objective of this study is to develop and test a predictive model for microbial removal in stormwater biofilters. This model should (1) represent the transport and fate of faecal microorganisms through biofilters; (2) include the governing processes for microbial removal; (3) reflect the influences of the key factors for microbial removal; (4) represent both wet weather events and dry periods, as these requirements are significant for the long-term prediction of the removal of faecal microbes and the optimization of biofilters. In addition, the model should be able to simulate different types of biofilter with various design (e.g. different plant types and media types). It should also be noted that, considering the complexity of microbial removal mechanisms, and data availability, complicated model structure may not always provide better results for microbial behavior modelling; alternatively, the uncertainty and stochasticity could be reflected in parameter values. Therefore, a parsimonious model with well-calibrated parameter values is favored, rather than an over-parameterized model. The model developed in this study will have a water flow module and a microbial quality module, and will be tested using the data collected from a 44 weeks long laboratory column experiments on five different configurations.

2. Methods

2.1. Model description

The model consists of two modules: the water flow module and the microbial quality module.

(1) Water flow module

The water flow module describes the major flow processes in biofilters. It was adapted from Randelovic et al. (2016) with some modifications. A "three-bucket" approach is employed, where the buckets represent the major parts of a biofilter: (1) the ponding zone (PZ) – a temporary pond on the top of the filter media, (2) the unsaturated zone (USZ) – the unsaturated filter media, and (3) the saturated/submerged zone (SZ) – the consistently saturated part of a biofilter created by a raised outflow pipe (Fig. 1). This method is simple in terms of computational requirement and coding difficulty (e.g. compared to the Richards equation), and it has been proved to be efficient enough to simulate the water flow in biofilters (Randelovic et al., 2016). The equations and parameters in the water flow module are listed in Tables 1 and 2-left, respectively.

In PZ, the water depth (h_p) is governed by inflow rate (Q_{in}), rainfall precipitation (Q_{rain}) and the infiltration to USZ (Q_{pf}) (Eq. (4)). Q_{pf} is determined by the hydraulic conductivity (K_s) and the degree of saturation in USZ (S) (Eq. (1)). S is assumed uniform over the entire USZ, and it is governed by Q_{pf}, evapotranspiration from USZ (Q_{et_usz}) and capillary rise (Q_{hc}) (Eq. (10)).

This paper uses a more comprehensive function to describe evapotranspiration compared to that implemented by Randelovic et al. (2016): the function introduced in FAO-56 (Eq. (6)) (Allen et al., 1998) was employed; the potential evapotranspiration for each plant type was calculated as the daily reference evapotranspiration (ET_0) that could be easily obtained from the Bureau of Meteorology for any Australian capital city (BOM, 2017), multiplied with a plant coefficient for evapotranspiration, K_c (Allen et al., 1998). This method considers the different water consumption ability between different plants, which was neglected by Randelovic et al. (2016). Moreover, Q_{et} is assumed to have impacts on both USZ and SZ, as the plant roots could adsorb water directly from both USZ and SZ, irrespective of capillary rise (Le Coustumer et al., 2012). This was also neglected by Randelovic et al. (2016). Therefore, Q_{et} is calculated as the degree of entire

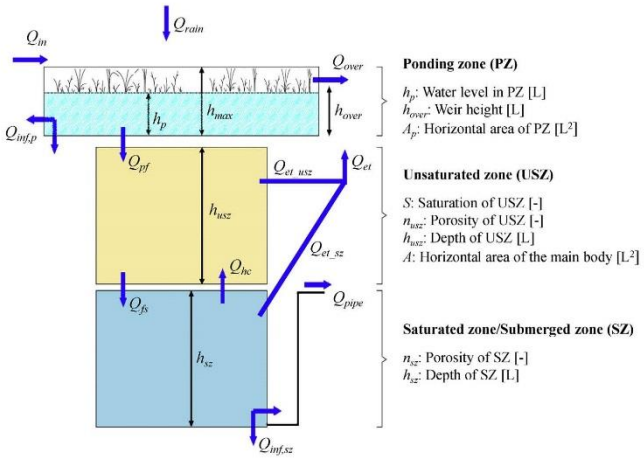


Fig. 1. Schematic representation of a typical stormwater biofilter and its flow scheme, and the key variables in each part (adapted from Randelovic et al. (2016) with modifications). Stormwater inflow (Q_{in}), rainfall precipitation (Q_{rain}), overflow (Q_{over}), water flow from PZ to USZ (Q_{pf}), flow from the USZ to SZ (Q_{fs}), evapotranspiration (Q_{et}; divided into the evapotranspiration from USZ (Q_{et_usz}) and from SZ (Q_{et_sz})), capillary rise (Q_{hc}), infiltration into the surrounding soil ($Q_{inf,p}$ and $Q_{inf,sz}$) and outflow (Q_{pipe}).

saturation in USZ plus SZ (S_{entire}) (Eq. (5)), and divided into Q_{et_usz} and Q_{et_sz} proportionally, according to the amount of water contained in USZ and SZ respectively (Eqs. (7) and (11)).

It is noted that, Eq. (6) was initially developed for vegetated biofilters; however, it was also used to test the unvegetated columns in this study, because the evaporation process for bare soil also yields the same trend described in Eq. (6) (i.e. a three-stage process based on the degree of saturation, as per Allen et al. (1998)).

The empirical function developed by Daly et al. (2009) was employed to calculate capillary rise (Q_{hc}) (Eq. (8)). The outflow rate (Q_{pipe}) is governed by the infiltration capacity of filter media (following Darcy's Law) as well as the level difference between SZ level and the height of SZ pipe (Eq. (13)). The water depth in SZ (h_{sz}) is controlled by the infiltration from USZ (Q_{fs}), Q_{hc}, and Q_{pipe}.

(2) Microbial quality module

The equations and parameters in the microbial quality module are listed in.

Tables 3 and 2-right, respectively. In Table 3, c_{in} in Eq. (14), c_p in Eq. (14), c_{usz} in Eqs. (15) and (16), and c_{sz} in Eqs. (19) and (20) respectively represent the microbial concentration in the water phase of inflow, ponding zone (PZ), unsaturated zone (USZ) and saturated zone (SZ). Adsorption, desorption and die-off of microbes are simulated as they are reported to be governing processes for microbial removal in biofilters (Chandrasena, 2014; Hathaway et al., 2011; Zhang et al., 2011).

Two different parameters are used here to represent adsorption and desorption instead of employing an equilibrium approach, because adsorption and desorption might be alternately dominant under different operational conditions. For example, adsorption may be dominant at times when new inflow has a high microbial concentration, while desorption plays a more important role when a flushing event (i.e. inflow with low microbial concentration) occurs after a normal event - the trapped microbes from the previous event could be flushed out due to desorption. Adsorption and desorption are assumed negligible in PZ, as the media in USZ and SZ has a far larger adsorption capacity than the plant foliage in PZ.

Die-off is assumed to occur throughout the biofilter (i.e. in PZ, USZ and SZ). During wet days, the inflow may remain in PZ for several hours before infiltrating into USZ; while during dry days, the microbes may be trapped in USZ and SZ from several hours to a few days, depending on the timing of the subsequent rainfall event.

Straining process is not included in this model, as initial sensitivity tests demonstrated that the model was as effective when straining was neglected as when straining was incorporated using the equation presented by Bradford et al. (2006). Although the importance of straining was reported (e.g. by Bradford et al. (2006) and Gargiulo et al. (2007)), these findings might not be transferable to the systems in this paper where different medias have been used, which may diminish the straining effect. To be more specific, microorganisms in biofilters exist either as free entities with a size that mostly ranges from 0.2 to 2 μm (Tortora et al., 1998), or associated with fine particulate matter (e.g. <6 μm according to Brown et al. (2013)); compared to these sizes, the 5th percentile media size in biofilters is much greater (>50 μm as per FAWB (2009)). As Updegraff (1983) reported that straining only becomes an important removal mechanism when the average bacteria size is greater than the 5th percentile media size, the effect of straining might be negligible in this study. Therefore, to avoid overparameterization, the straining process is not incorporated in this model. However, it is noted that the straining equations (like that presented by Bradford et al. (2006)) can easily be incorporated into the model structure if necessary.

Sedimentation and resuspension in PZ are also neglected, because (1) the sedimentation velocity of *E. coli* in stormwater (0.3–1.0 m/day according to Cizek et al. (2008)) is very low when compared with the infiltration velocity, and (2) only minimum turbulent velocity fluctuations occur in PZ of a biofilter, hence resuspension is not likely to occur.

The microbial quality module also simulates the transport and fate of microbes using the "three-bucket" approach. The adsorption rate (k_{att}),

Table 1
Equations for the water flow module.

Water flow module equation	Eq. no.
General form of equations	
$Flow = \min$ (physically possible; available upstream; available downstream)	
Ponding zone (PZ)	
Infiltration from PZ to unsaturated zone	
$Q_{pf} = \min(K_s A \frac{h_p + h_{usz}}{h_{usz}}, \frac{h_p A_p}{dt} + Q_{in} + Q_{rain}, \frac{1}{dt}(1-S) n_{usz} h_{usz} A)$	(1)
Infiltration from PZ to the surrounding soil	
$Q_{inf,p} = \begin{cases} \min(K_f[(A_p - A) + C_s h_p P_p], \frac{h_p A_p}{dt}), \text{if unlined} \\ 0, \text{if lined} \end{cases}$	(2)
Overflow through weirs	
$Q_{over} = \begin{cases} \min(C_Q B \sqrt{2g(h_p - h_{over})}^3, \frac{A_p(h_p - h_{over})}{dt}), h_p > h_{over} \\ 0, h_p \le h_{over} \end{cases}$	(3)
Water mass balance in PZ	
$\frac{d(h_p A_p)}{dt} = Q_{in} + Q_{rain} - Q_{pf} - Q_{over} - Q_{inf,p}$	(4)
Unsaturated zone and saturated zone (USZ and SZ)	
Entire Saturation in USZ plus SZ	
$S_{entire} = \frac{S \times n_{usz} h_{usz} + n_{sz} h_{sz}}{n_{usz} h_{usz} + n_{sz} h_{sz}}$	(5)
Total Evapotranspiration from USZ and SZ	
$Q_{et} = \begin{cases} 0, S_{entire} \le S_w \\ A \times K_c \times ET_0 \frac{S_{entire} - S_w}{S_s - S_w}, S_w < S_{entire} \le S_s \\ A \times K_c \times ET_0, S_s < S_{entire} \le 1 \end{cases}$	(6)
Unsaturated zone (USZ)	
Evapotranspiration from USZ	
$Q_{etusz} = Q_{et} \times \frac{S \times n_{usz} h_{usz}}{S \times n_{usz} h_{usz} + n_{sz} h_{sz}}$	(7)
Flow due to capillary rise	
$Q_{hc} = AC_r(S - S_s)(S_{fc} - S), C_r = \frac{4 \times K_s \times ET_0}{2.5(S_{fc} - S_s)^2}$	(8)
when $S_s \le S \le S_{fc}$, otherwise $Q_{hc} = 0$	
Infiltration from USZ to SZ	
$Q_{fs} = \begin{cases} \min(A \times K_s \frac{h_p + h_{usz}}{h_{usz}} S^\gamma, \frac{(S - S_{fc}) A \times n_{usz} h_{usz}}{dt} + Q_{pf} + Q_{hc}), S \ge S_{fc} \\ 0, S < S_{fc} \end{cases}$	(9)
Water mass balance in USZ	
$\frac{d(S \times n_{usz} h_{usz} A)}{dt} = Q_{pf} + Q_{hc} - Q_{fs} - Q_{etusz}$	(10)
Saturated zone (SZ)	
Evapotranspiration from SZ	
$Q_{etsz} = Q_{et} \times \frac{n_{sz} h_{sz}}{S \times n_{usz} h_{usz} + n_{sz} h_{sz}} = Q_{et} - Q_{etusz}$	(11)
Infiltration from SZ to the surrounding soil	
$Q_{inf,sz} = \begin{cases} \min(K_f(A + C_s P_{sz} h_{sz}), \frac{h_{sz} h_{sz} A}{dt}), \text{if unlined} \\ 0, \text{if lined} \end{cases}$	(12)
Flow through drainage pipe	
$Q_{pipe} = \begin{cases} \min(A \times K_s \frac{h_p + h_{usz}}{h_{usz} + h_{sz}}, \frac{(h_{sz} - h_{pipe}) n_{sz} A}{dt} + Q_{fs} - Q_{hc} - Q_{etsz} - Q_{inf,sz}), h_{sz} > h_{pipe} \\ 0, h_{sz} \le h_{pipe} \end{cases}$	(13)

desorption rate (k_{det}) and the standard die-off rate (μ_0) are all assumed to be the same in both the USZ and SZ. However, the model is flexible enough to allow different segments of a biofilter to have different k_{att}, k_{det} and μ_0 (according to Stott et al. (2017), poorer microbial retention of saturated in comparison to unsaturated conditions has been reported in literature), but this is likely only efficient when sufficient experimental data exist to demonstrate their differences. Furthermore, initial sensitivity tests using depth-disaggregated parameter values did not produce better prediction than using depth-averaged ones.

The transport of microbes in the USZ and SZ are modelled with one-dimensional advection-dispersion equation (Eqs. (15) and (19)), where M_1 and M_2 are the microbial concentration in the solid phase due to adsorption in USZ and SZ respectively. The value of dispersivity (λ) in Eqs. (17) and (21) was obtained from the results of tracer studies.

Similar to the majority of published microbial models (Chandrasena et al., 2013; Zhang et al., 2010; Zhang et al., 2012), this model also uses first-order kinetics equations (Eqs. (16), (20) and (23)) to present governing processes. Temperature (T) is incooperated in die-off (Eq. (23)) since it is a crucial factor for the growth/die-off of microbes (Chandrasena et al., 2014).

2.2. Data used for model testing

The model was tested with the data collected from laboratory column experiments conducted in a greenhouse at Monash University (Australia) over 44 weeks during 2012 and 2013. Five biofilter configurations (five replicates each) with two different media types and three plant types were selected to test the model (Table 4). These media and plant types were selected due to their wide application as a result of their effectiveness in microbial and/or nutrient removal (Chandrasena et al., 2014; FAWB, 2009). All the configurations are with the same structure (i.e. diameter, depth of each part); only the media type and/or plant type are different between configurations.

Full details of the system setup and sampling can be found in Chandrasena et al. (2017), while only a summary is provided here for context. Semi-natural stormwater was used for dosing, which was made in the laboratory by mixing sediments from stormwater ponds, chemicals, raw sewage and dechlorinated tap water to target the "average" pollutant concentrations based on a worldwide review of stormwater quality conducted by Duncan (1999) (for more details of stormwater preparation please refer to Chandrasena et al. (2017) and Bratieres et al. (2008)). In general, each biofilter was dosed twice

Table 2
Parameters in the model. Input parameters: parameters based on design and measurement. Calibration parameters: parameters calibrated in this study.

Water flow module parameters	Microbial quality module parameters
Input parameters	
B Length of overflow weir [L]	ρ Bulk soil density [$M L^{-3}$]
P_p Unlined perimeter [L]	λ Dispersivity [L]
C_Q Weir overflow coefficient [−]	
C_s Side infiltration coefficient [−]	
K_f Hydraulic conductivity of the surrounding material [$L T^{-1}$]	
S_w Wilting point [−]: washed sand, 0.05[a]; loamy sand, 0.07[a]	
S_s Saturation as the threshold for plants to reach potential evapotranspiration [−]: without SZ, 0.22[b]; with SZ, 0.37[b]	
S_{fc} USZ saturation at field capacity [−]: without SZ, 0.37[b]; with SZ, 0.61[b]	
γ Relative hydraulic conductivity coefficient dependent on soil type [−]: washed sand, 11.1[c]; loamy sand, 11.76[c]	
Calibration parameters	
K_s Hydraulic conductivity of the filter media [$L T^{-1}$]	k_{att} Adsorption rate [T^{-1}]
K_c Plant coefficient for evapotranspiration [−]	k_{det} Desorption rate [T^{-1}]
	μ_0 Standard die-off rate at given reference conditions (e.g. standard temperature) [T^{-1}]
	θ Temperature correction coefficient for die-off [−]

[a] Allen et al. (1998).
[b] Randelovic et al. (2016).
[c] Dingman (2002).

Table 3
Equations for the microbial quality module.

Microbial quality module equation	Eq. no.
Ponding zone (PZ)	
Microbial mass balance in PZ	
$\frac{d(c_p h_p A_p)}{dt} = c_{in} Q_{in} - c_p (Q_{pf} + Q_{over} + Q_{inf,p}) - \mu c_p h_p A_p$	(14)
Unsaturated zone (USZ)	
Microbial mass balance in the water phase	
$\frac{\partial(Sn_{usz} c_{usz})}{\partial t} + (Sn_{usz} k_{att} c_{usz} - \rho k_{det} M_1) = \frac{\partial}{\partial z}(Sn_{usz} D_1 \frac{\partial c_{usz}}{\partial z}) - \frac{\partial(q_1 c_{usz})}{\partial z} - Sn_{usz} \mu c_{usz}$	(15)
Adsorption, desorption and die-off of adsorbed microbes in the soil phase	
$\frac{\partial M_1}{\partial t} = \frac{n_{usz} S}{\rho} k_{att} c_{usz} - k_{det} M_1 - \mu M_1$	(16)
Dispersion coefficient in USZ	
$D_1 = \lambda \frac{q_1}{n_{usz} S}$	(17)
Average unit flow in USZ	
$\bar{q}_1 = \frac{\alpha_1 (Q_{pf} - Q_{et}) + \beta_1 (Q_k - Q_{uc})}{A}$	(18)
where $\alpha_1 + \beta_1 = 1$, and $\alpha_1 = 1$ at upper boundary, $\beta_1 = 1$ at lower boundary	
Saturated zone (SZ)	
Microbial mass balance in the water phase	
$\frac{\partial(n_{sz} c_{sz})}{\partial t} + (n_{sz} k_{att} c_{sz} - \rho k_{det} M_2) = \frac{\partial}{\partial z}(n_{sz} D_2 \frac{\partial c_{sz}}{\partial z}) - \frac{\partial(q_2 c_{sz})}{\partial z} - n_{sz} \mu c_{sz}$	(19)
Adsorption, desorption and die-off of adsorbed microbes in the soil phase	
$\frac{\partial M_2}{\partial t} = \frac{n_{sz}}{\rho} k_{att} c_{sz} - k_{det} M_2 - \mu M_2$	(20)
Dispersion coefficient in SZ	
$D_2 = \lambda \frac{q_2}{n_{sz}}$	(21)
Average unit flow in SZ	
$\bar{q}_2 = \frac{\alpha_2 (Q_k - Q_{uc}) + \beta_2 (Q_{pgw} + Q_{inf,sz})}{A}$	(22)
where $\alpha_2 + \beta_2 = 1$, and $\alpha_2 = 1$ at upper boundary, $\beta_2 = 1$ at lower boundary	
Die-off rate in each part	
$\mu = \mu_0 \theta^{T-20^\circ C}$	(23)

weekly with 13 L of stormwater, which represents the average rainfall event size in Melbourne when assuming the biofilters are sized to 2% of their impervious catchment area (FAWB, 2009).

During the monitoring periods, 16 sampling events (i.e. when inflow and outflow samples were taken) were used to assess the performance of the biofilters. In most sampling events, 20 L of stormwater was placed into each biofilter. This volume represents the 1 in 1 month average recurrence interval (ARI) event for Melbourne (equivalent loading of 8.84 mm per event). To represent the natural variability observed in the field, sampling events were further varied in their characteristics. Three sampling events were dosed with double water volume (40 L) to simulate high loading events (equal to a 1 in 3 month ARI event; equivalent loading of 17.68 mm per event). To evaluate the influence of long dry periods on systems' performance, four events were implemented after periods of at least one week without dosing. One flushing event was used to study desorption effects, where the columns were dosed with 20 L of clean water. In this way, various operational conditions were included – the *Escherichia coli* (*E. coli*) concentrations in the inflow: 185–42,227 MPN/100 mL; the antecedent dry days: 1 day–6 weeks: the dosing rates: 20 L and 40 L. In addition, in the last three sampling events, infiltration rates of three replicates of each configuration except CP were restricted to 48 mm/h by controlling the outflow pipes, to study the influence of infiltration rate on microbial removal.

The outflow samples of each column were collected in two batches: (1) "old water" – water that remained in the SZ after the previous dry period, which could be comparatively clean, having had sufficient time for die-off to occur (Chandrasena et al., 2014) and (2) "new water" – water that has passed through the column in the current event. Tracer studies suggested that the typical volume of water in SZ (i.e. "old water") was 10 L (please see the supplementary materials of Chandrasena et al. (2017) for more details on tracer test).

For each replicate, there were 16 composited inflow samples (one per event) and 31 to 35 outflow samples (including composited "old water" and composited "new water" during each event). In addition, discrete outflow samples were collected for some replicates during some events; in these events, effluent was sampled every two liters of outflow.

As per various other studies (Chandrasena et al., 2014; Chandrasena et al., 2014), *Escherichia coli* (*E. coli*) was used as an indicator of microbial behavior in biofilters. All samples were transported on ice to the Environmental and Public Health Microbiology Laboratory (EPHM Lab) for enumeration of *E. coli* using the Colilert method™ (IDEXX-Laboratories, 2007).

During sampling events, instantaneous water levels in PZ were recorded at a regular interval (3 min and 45 s) using rulers that were fixed to the wall of each column's PZ. For different replicates, the number of total valid measurements of water level in PZ varies from 124 to 141. Total effluent volumes were also measured using individual outflow collection tanks for all columns during all sampling events.

2.3. Methods for model testing

2.3.1. Calibration and sensitivity analysis

The two modules in the model were calibrated separately. The Nash-Sutcliffe Efficiency Criteria, E (Nash and Sutcliffe, 1970), was used to calibrate and evaluate model performance.

(1) Water flow module

In this module, two parameters were calibrated: (1) hydraulic conductivity, K_s and (2) the plant coefficient for evapotranspiration, K_c. Both K_c and K_s could vary between replicates because of the different operational conditions that inherently occur in each system (e.g. clogging, cracks and plant growth rates). As such, the calibration of these parameters was performed for each column individually. This allowed obtainment of accurate hydrodynamics so that the flow predictions would not confound microbial concentration modelling.

The parameter sets were generated based on the full combination of K_s and K_c with incremental steps: K_s ranged from 50 to 500 mm/h (FAWB, 2009), with an increment step of 1 mm/h; K_c ranged from 0.1 to 5.0 (Allen et al., 1998), with an increment step of 0.1; all the combinations of K_s and K_c values were tested (22,550 parameter sets in total). For each parameter set, the water flow module was run using the inflow rates, and wet and dry weather patterns during the experiments. Outflow rates and ponding depths (i.e. water levels in PZ) were recorded for each parameter set.

It is noted that, since Q_{pf} (the infiltration rate from the PZ to USZ, Eq. (1) in Table 1) in the last three events was fixed at 48 mm/h, K_s was only calibrated according to the data from first 13 events. In this way, the calibrated K_s could reflect the "natural" characteristic (i.e. without control) of biofilters.

K_s and K_c were calibrated using an equal weighted multi-objective function of ponding depths and event outflow volumes (Eq. (24)) (Caramia and Dell'Olmo, 2008). This was to balance the calibration of K_s (mainly represented by ponding depths) and K_c (mainly represented by outflow volumes).

$$E_q = 0.5 \times (E_{\text{depth}} + E_{\text{volume}}) \tag{24}$$

where E_q is the average E for the water flow module, E_{depth} is the E value calculated using the measured and predicted ponding depths (for K_s), and E_{volume} is the E value calculated using measured and predicted event outflow volumes (for K_c).

In the simulation of this module, the time step (dt) was set as 1 min.

(2) Microbial quality module

The method of Vezzaro et al. (2013) was utilized for the calibration and sensitivity analysis of the microbial quality module. This method is known to reduce the subjectivity of traditional Monte-Carlo methods.

Table 4
Characteristics of the biofilters studied in the laboratory. PZ: ponding zone; USZ: unsaturated zone; PZ: saturated zone.

	Plant type	Media type in USZ	Media type in SZ	Depth of each part	Diameter of column
WS	No plant	Washed sand	Washed sand (300 mm) + coarse sand (70 mm) + gravel (70 mm)	PZ: 280 mm; USZ: 400 mm; SZ: 440 mm	240 mm
LS	No plant	Loamy sand			
PB	*Palmetto buffalo*	Washed sand			
LC	*Leptospermum continentale*	Washed sand			
CP	*Carex appressa*	Washed sand			

In this module, four parameters were calibrated: k_{att}, k_{det}, θ, and μ_0. These were calibrated simultaneously for all five replicates of each configuration, as the model assumes that the differences of these parameter values between replicates of the same configuration were negligible and the water quality data did not support individual column-by-column calibration.

100,000 parameter sets were randomly generated and the initial range of each parameter was set based on what was physically possible and the values obtained from literature or experiments. k_{att}: 0.1–6 h^{-1}, uniformly distributed; k_{det}: 10^{-5}–4 h^{-1}, log uniformly distributed (*Log* (k_{det}): −5–0.6 h^{-1}); θ: 0.9–1.6, uniformly distributed; and μ_0: −1–4 day^{-1}, uniformly distributed. For each parameter set, the microbial quality module was run using the flow conditions obtained from the calibrated water flow module and microbial loadings from the measured inflow datasets, and the outflow *E. coli* levels were recorded.

All the effluent *E. coli* concentrations were log-transformed before being used to calculate *E* values. This was because *E. coli* levels often yield lognormal distributions (NHMRC, 2008). Moreover, the outflow concentrations varied significantly (60–18,000 MPN/100 mL); the use of log-transformed concentrations meant that the peaks would not be overemphasized and cause bias, as *E* favors peaks (Criss and Winston, 2008).

In the simulation of this module, the time step (dt) was set as 1 min, and the space step (dz) was set as 40 mm.

2.3.2. Validation

Validation was conducted on the microbial quality module to determine how the model performs outside of its calibration dataset. A thorough splitting exercise was conducted for each configuration, following the methodology introduced by Mourad et al. (2005) and McCarthy (2008): (1) *n* events are randomly selected from the 16 sampling events; (2) the model's parameters are calibrated using the selected *n* events following the methods developed by Vezzaro et al. (2013); (3) the model is run using the calibrated parameter sets, the outputs for the *n* calibrated events and for the remaining (16 - *n*) events (validation events) are recorded; (4) Steps (1) to (3) are repeated up to 500 times; and (5) n is incremented by one and Steps (1) to (4) are repeated until $n = 17$.

During these steps the Root Mean Square Error (RMSE) was calculated for model evaluation instead of *E*, because (1) RMSE is proportional *to E*, and (2) as opposed to *E*, RMSE values are always positive allowing the outputs to be plotted using logarithmic axes without transformations.

To find the optimum splitting ratio for each configuration, all calibration distributions were compared with their respective validation distributions using Wilcoxon Rank Sum Tests (McCarthy, 2008; Mourad et al., 2005). The output is a significance probability of *p* for each splitting ratio; where a value of $p > 0.05$ means the calibration and validation results yield similar model performances, and therefore the model is adequately calibrated.

3. Results and discussion

3.1. Calibration of water flow module

Table 5 summarizes the calibration results for the water flow module. A high E_q was achieved for each replicate, ranging from 0.82 to 0.95. Compared to the vegetated configurations (PB, LC and CP), the

Table 5
Calibrated K_s and K_c and the corresponding Nash-Sutcliffe coefficients (*E*) for each replicate of each configuration. E_{depth} is the model performance in predicting infiltration rates; E_{volume} is the model performance in predicting outflow volumes; E_q is the overall model performance based on the multi-objective calibration.

Configuration		Replicate1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
WS	K_s (mm/h)	182	199	153	178	170
	E_{depth}	0.87	0.92	0.81	0.87	0.79
	K_c (−)	0.4	0.5	0.5	0.4	0.4
	E_{volume}	0.98	0.98	0.98	0.97	0.96
	E_q	0.93	0.95	0.90	0.92	0.87
LS	K_s (mm/h)	106	118	104	99	118
	E_{depth}	0.92	0.89	0.91	0.90	0.92
	K_c (−)	0.3	0.3	0.4	0.3	0.2
	E_{volume}	0.98	0.93	0.94	0.99	0.97
	E_q	0.95	0.91	0.92	0.94	0.94
PB	K_s (mm/h)	170	134	146	172	127
	E_{depth}	0.91	0.80	0.88	0.89	0.84
	K_c (−)	0.7	0.6	0.7	0.6	0.6
	E_{volume}	0.98	0.97	0.98	0.97	0.97
	E_q	0.94	0.89	0.93	0.93	0.91
LC	K_s (mm/h)	156	144	149	102	150
	E_{depth}	0.90	0.80	0.87	0.81	0.85
	K_c (−)	1.3	1.6	1.2	1.1	1.4
	E_{volume}	0.98	0.94	0.96	0.95	0.94
	E_q	0.94	0.87	0.91	0.88	0.90
CP	K_s (mm/h)	137	138	96	105	100
	E_{depth}	0.86	0.81	0.75	0.81	0.78
	K_c (−)	1.3	1.4	1.1	1.2	1.0
	E_{volume}	0.97	0.96	0.88	0.93	0.89
	E_q	0.92	0.89	0.82	0.87	0.83

unvegetated configurations (WS, LS) have generally high E_q, probably because the existence of plants introduced additional complexity in water uptake processes that were not fully captured by the model.

E_{volume} ranges from 0.88 to 0.99, suggesting an excellent fit between modelled and measured outflow volumes. In addition, only minor errors were found between modelled and measured total outflow volumes for each column: for WS replicates, 1.53%–3.55%; for LS replicates, 1.79%–5.87%; for PB replicates, 0.37%–3.33%; for LC replicates, 3.21%–6.09%; and for CP replicates, 3.10%–6.08%. These results also confirm that the antecedent SZ levels could be well predicted, as the outflow volume is inherently dependent on the remaining SZ volume. Only slight differences in K_c values were found between-replicates of a specific configuration; however, K_c varied considerably between configurations, highlighting the influence of plant and media types on evapotranspiration. The evapotranspiration rates for unvegetated configurations are, on average, 65% lower than the vegetated configurations, since there is no transpiration from bare soils. The evapotranspiration rates for LC and CP are higher than PB (averaged K_c value: 0.64 for PB, 1.32 for LC, 1.2 for CP), reflecting the plant and root sizes and hence their ability to transpire water (Le Coustumer et al., 2012).

E_{depth} ranges from 0.75 to 0.92, and 88% of them are above 0.80. The calibrated K_s range obtained through calibration (96 mm/h to 192 mm/h) agrees with practical values suggested by various guidelines (e.g. 100–300 mm/h according to FAWB (2009)). The high between-replicate variation of K_s for a given configuration (e.g. replicate 4 of LC vs. other LC replicates) agrees with the findings in literature, and reflects the random nature of clogging and associated processes (Le Coustumer et al., 2012). K_s varied considerably between configurations, reflecting the influences of media type and plant type on hydraulic conductivity. The average higher K_s for WS (176.4 mm/h) compared to LS (109 mm/h) is in response to the higher clay content in the latter, which results in lower infiltration rates and higher possibility of clogging (FAWB, 2009; Le Coustumer et al., 2012). The lower averaged K_s values for vegetated systems (149.8 mm/h for PB, 140.2 mm/h for LC, 115.2 mm/h for CP) compared to WS could be due to some of the pores being occupied by roots. Although Hatt et al. (2007) observed that with time the plants with extensive roots reinstated the infiltration rate through the creation of macropores by swelling and shrinking of roots, changes in hydraulic conductivity was not captured in this module. This does not appear to hinder model function, as high values of E_{depth} suggest that the unchanged K_s is adequate for modelling flow processes.

To further explore the water flow module's performance, the modelled outflow rates of LC3 (replicate 3 of LC, a representative for vegetated columns with poor prediction) and WS2 (replicate 2 of WS, a representative for unvegetated columns with good prediction) were plotted with the measured values in Event 11 (where discrete samples were collected and hence used to provide discrete measurements of outflow rates with time over the event; Fig. 2). The measured values were obtained by dividing the water volume of each discrete sample (2 L) by the time taken to collect that sample. For both columns, the measured and modelled outflow rates fitted well, especially considering the discrete sample data were not used for model calibration. However, the modelled outflow rates of LC3 were generally higher than measured ones. This is because the hydraulic conductivity of LC3 was more variable compared to that of WS2 due to plant growth/clogging; it is very possible that the calibrated K_s for LC3 based on all events overestimated the practical K_s in Event 11 (e.g. clogging might occur after operating for more than half a year).

3.2. Results of microbial quality module

3.2.1. Calibration

All the calibrated values of the optimum parameter sets fall into the ranges that were obtained from literature (Table 6). However, the value of each individual parameter varies between different configurations. This could be a result of the design of different configurations having different impacts on each governing process. For example, compared to the washed sand, loamy sand has a higher content of clay; therefore, the media of LS is less stable and more likely to detach from the column when water passes through (FAWB, 2009); during this process, microbes may be drained out after becoming associated with the detached media. The higher calibrated desorption rate of LS compared to WS in Table 6 demonstrated the phenomena. In addition, the lower die-off rate of LS compared to WS agrees with the findings of Ferguson et al. (2003); that is because loamy sand contains higher nutrient content, it is less effective at inactivating microbes when compared with washed sand. Furthermore, the variability of both die-off and adsorption rates between different plants also agree with the results of Chandrasena et al. (2014), who found vegetation type affects treatment. The higher adsorption rates and lower desorption rates of LC and PB compared to CP in Table 6 are also in agreement with the literature in *Leptospermum continentale* and *Palmetto buffalo* are more effective for microbial removal than *Carex appressa* (Chandrasena et al., 2014). While calibrated results largely agree with findings reported in the literature, some disagreements are also found. For instance, loamy sand has been reported as being more effective than washed sand in retaining microbes (Ferguson et al., 2003), however, the calibrated adsorption rate of LS is

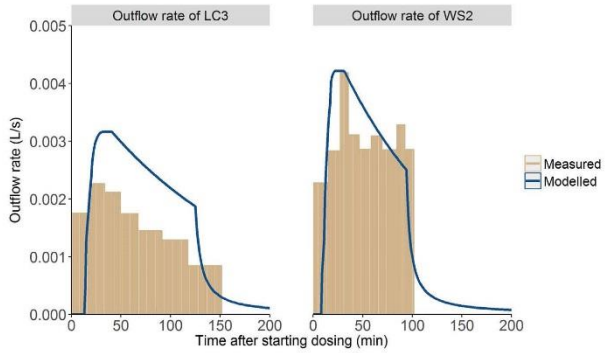


Fig. 2. Modelled and measured outflow rates of LC3 and WS2 during Event 11. The blue lines represent the modelled values in each minute/time step, while the bar charts with yellow color represent the measured values in each minute/time step. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 6
Parameter values in the optimum parameter set for each configuration, their corresponding E_c, and parameter values reported in literature.

	E_c	k_{att} (h^{-1})	Log (k_{det}) (h^{-1})	θ (−)	μ_0 (day^{-1})
WS	0.64	2.74	−0.35	1.07	0.61
LS	0.68	1.35	−1.31	0.98	0.25
PB	0.63	2.48	−0.04	1.13	1.11
LC	0.56	1.76	−0.53	1.01	0.03
CP	0.46	1.46	−3.12	1.14	0.64
Literature values		0.20–5.86[a,b]	−4.22–0.31[a,b]	0.1.01–1.19[c]	0.06–1.23[d,e]

[a] Bradford et al. (2006).
[b] Gargiulo et al. (2008).
[c] Brauwere et al. (2014).
[d] Chandrasena (2014).
[e] Crane and Moore (1986).

lower than WS in Table 6. This is a result of correlations/compensations between parameters that occurred during calibration, which led to the overestimation and/or underestimation of parameter values. An explanation of parameter correlations/compensations is explored using the sensitivity testing conducted in the next section.

Comparison of measured and modelled outflow concentrations indicates good agreement (Fig. 3), as the majority of points in Fig. 3 are scattered around the 1:1 line and generally high E_c values were obtained (0.46 to 0.68, in Table 6). These E_c values compare favorably to other microorganism models: e.g. in a model for microorganism prediction in urban stormwater (MOPUS), E_c ranged from 0.25 to 0.41 (McCarthy et al. (2011)). Although slightly higher E_c (0.53–0.86) were achieved in the biofilter model developed by Chandrasena et al. (2013), a smaller dataset was used to calibrate that model. Moreover, the simplicity of that model meant that it could neither represent the concentrations inside biofilters nor the influences of temperature. Considering the capability of the model developed in this study, and the limited data used for calibration, the E_c obtained here represent good model performance.

On average, the module generally provided better prediction for the unvegetated configurations (i.e. WS and LS), and progressively worsened for configurations with denser vegetation/root systems (PB > LC > CP). One explanation is that plants introduce complex microbial removal processes, the finite details of which are not catered for by the model. For example, while some studies suggest that the rhizosphere (region near plant roots) can harbor microorganisms (Mukerji et al., 2006), others have suggested the opposite due to antimicrobial root exudation (Bais et al., 2006; Strehmel et al., 2014) and the macropores created by plant roots (Hathaway et al., 2011; Rusciano and Obropta, 2007). Moreover, this model does not capture the dynamic nature of the biofilters. In fact, during the 44-week-long experiments, significant changes would have occurred in each system, including plant growth and clogging. As only static (i.e. not time-dependent) parameters were used, these temporal changes were not captured.

Fig. 4 indicates that the microbial concentrations in the outflow could also be well predicted for LC3 and WS2 during Event 11. However, the modelled peak for both LC3 and WS2 were underestimated by the model, which is due to the high variability of microbial concentration within an event (over 30% according to McCarthy et al. (2008)). In addition, the predicted peaks occur slight later than the measured peaks. This might be because the dispersivity values, obtained from tracer studies conducted before Event 1, do not adequately represent dispersivity during Event 11, due to the dynamic nature of these systems. Better predictions are expected if more accurate dispersivity values are available.

3.2.2. Sensitivity analysis

Sensitivity analysis for each configuration was conducted. Since the patterns of parameter distributions for each configuration are very similar, only the results for one vegetated configuration, LC, are shown in Fig. 5 as an example; results for the other four configurations are presented in Appendix A.

The peaked distributions obtained for k_{att} and k_{det} (Fig. 5) suggest that adsorption and desorption are the two dominant processes for microbial removal, as a clear peak/flat shape in a parameter distribution indicates that the model is sensitive/insensitive to that parameter (Dotto et al., 2012). This conclusion fits the results obtained from another model of microbial removal in biofilters (Chandrasena et al., 2013). However, the model performance is not very sensitive to θ and μ_0, which contradicts the previous findings that die-off is also an important process for microbial removal, and temperature is the key factor for die-

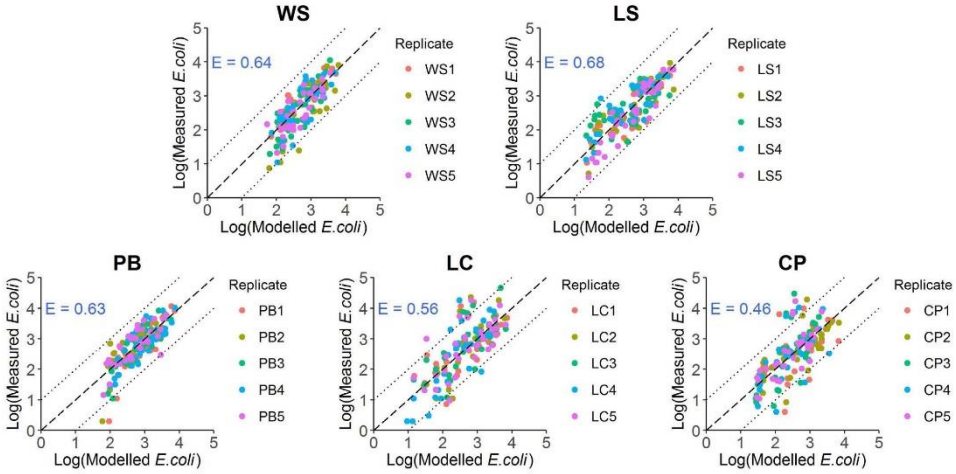


Fig. 3. Comparison of measured and modelled outflow concentrations. Dashed lines indicate the 1:1 line between modelled and measured *E. coli* concentrations, while dotted lines indicate error bars (+/− one order of magnitude).

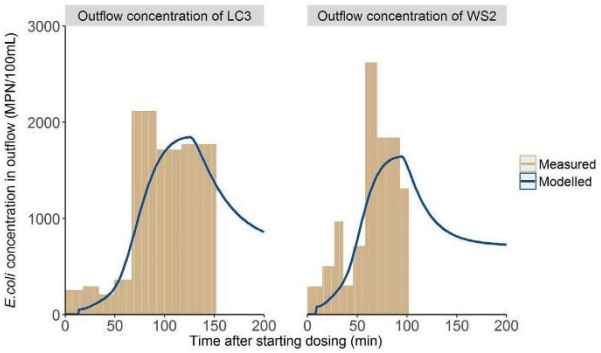


Fig. 4. Modelled and measured outflow concentrations of LC3 and WS2 during Event 11. The blue lines represent the modelled values in each minute/time step, while the bar charts with yellow color represent the measured values in each minute/time step. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

off (Chandrasena et al., 2014; Stevik et al., 2004). It is hypothesized that the data used for calibration is insufficient to interpret the importance of die-off (θ and μ_0): die-off is only dominant during dry days, and its effects may only be fully revealed with sufficiently long dry periods. However, only four of the 16 sampling events were conducted after dry periods; the limited data reflecting die-off effects has resulted in the model not being sensitive to θ and μ_0.

Correlations between different parameters were found, suggesting that different parameter combinations can lead to the same results, because they could compensate for each other (Dotto et al., 2012). The correlations could be due to data availability: e.g. flushing was the main experiment that could reveal desorption; however, there was only one flushing event in the 16 sampling events. Hence, k_{det} could not be adequately calibrated, and correlations between k_{det} and other parameters were found. For example, since adsorption and desorption are "opposite" processes, the value of k_{att} has to increase/decrease to compensate the increment/decrement of the value of k_{det}. Similar trends were also found between k_{det} and θ/μ_0. These results could explain the big differences of k_{det} values between different configurations in Table 6, and high k_{det} value always accompanied with high k_{att} and/or μ_0 value.

3.2.3. Validation

Boxplots for the validation results of WS and PB (one unvegetated and one vegetated) were selected as examples (Fig. 6); the results for other three configurations are presented in Appendix A.

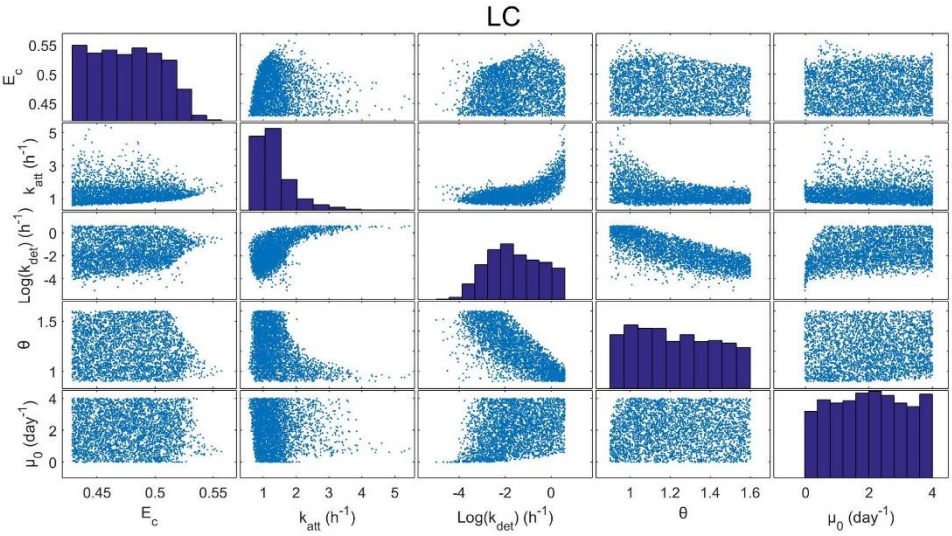


Fig. 5. Matrix plot of LC for sensitivity analysis. The diagonal histograms represent the distributions of E_c and all the parameters; the scatter plots between parameters reveal the parameter interactions.

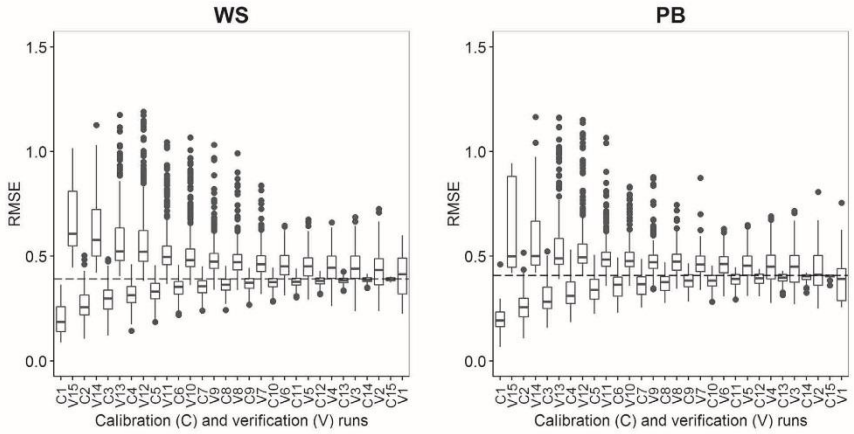


Fig. 6. Results of the detailed calibration/validation analyses (WS and PB). *Cn*: the calibration results when randomly choosing *n* events for calibration; V16-*n*: the validation results when the model is validated with the remaining (16 - *n*) events. The dotted horizontal line represents the optimum RMSE values for each configuration when the model is calibrated with all 16 events.

For calibration datasets (Fig. 6), when the number of events for calibration (n) is small (e.g. C1), the median values of RMSE were well below the dotted line, as the possibility of accurately predicting events' *E. coli* levels is high; in the meantime, high between-event variability made the model's performance vary dramatically. As n increased, the median RMSE value increased, and variability of the RMSE value decreased as the calibration represented the majority of the event characteristics. The trend continued, with the median RMSE increasing until very close to the RMSE value of the model when calibrated with all 16 events (the dotted line).

For validation datasets (Fig. 6), the median RMSE value decreased as the number of validation events decreased. As discussed before, using more events for calibration could cover a wider range of possible event characteristics, meaning that the model is more likely to perform better during validation. This could also explain the initial decrease of RMSE values' variability for validation. However, after a certain point, the variability of RMSE values increased again as the number of validation events decreased. That is because, when validating with a reasonable number of events, the errors in different events might compensate with each other, hence the RMSE values were relatively consistent; however, this compensation is less likely to occur when the number of validation events is small, especially when the validation events contained only special characteristics (e.g. flushing event) - this produced highly variable RMSE values.

For LS and PB, the calibration and validation distributions were deemed similar only after having used 14 or 15 events for calibration (Table 7). This relatively high data requirement is not uncommon for stormwater quality models, with both Mourad et al. (2005) and McCarthy (2008) highlighting this issue.

Table 7
Number of events required to adequately calibrate the model and their corresponding significance probabilities (p).

Configuration	Number of events required for adequate calibration	Percentage of total events for adequate calibration	Significance probabilities (p) or highest p value
WS	–	–	6.83E-6
LS	14	88%	0.59
PB	15	94%	0.17
LC	–	–	7.69E-17
CP	–	–	5.70E-54

The cause for the high number of events required for calibration could be: (1) some temporal factors (e.g. long-term clogging processes) were not captured by the model, hence a balanced mix of early and late events were required in both calibration and validation datasets, (2) over 50% of the events were extreme in nature (high volume, dry weather, flushing, controlled outlet, etc.), thereby enough representation of such events was required to adequately estimate the model's parameters during calibration, and/or (3) large uncertainties were associated with *E. coli* concentrations (McCarthy et al., 2008), resulting in high data requirements to compensate for these uncertainties.

For the other three configurations (WS, LC and CP), the model's performance during validation was never similar or better than what was observed during calibration. This means the model's parameters could not be adequately estimated even when calibrated with most events. This was especially the case for the two well-vegetated systems (CP and LC), and is likely explained not only by the above factors, but also by the fact that the model was unable to represent the dynamic nature of plant growth.

4. Conclusions

This paper presented a process-based model for microbial removal in stormwater biofilters. This model includes three key processes and an operational factor that govern the behavior of microbes in biofilters.

Compared to the previous models developed in this field, this model could achieve a long-term simulation under various weather patterns. In addition, this model is capable of reflecting the differences in evapotranspiration between different plants, enabling it to be applied to various designs.

By testing the model with 44-week-long laboratory experiments on five different biofilter configurations, the predicted results showed good agreement with the measured data. A comparison of the Nash-Sutcliffe (E) values achieved in this model for microbial removal (0.46–0.68) and the E reported in other literature indicated that this model is very promising. In addition, the calibrated model parameter values fitted those reported in literature very well.

The sensitivity analyses results indicated that adsorption and desorption processes were dominant; however, due to insufficient data available for calibration, correlations between different parameters were found. The validation results further demonstrated that more data are required to adequately calibrate the model. Therefore, it is suggested that the model be validated with a larger dataset.

Acknowledgements

This study was partly financed by the Australian Research Council – Discovery Early Career Researcher Award (Grant number DE140100524). The authors also wish to acknowledge the support of Dr. Edoardo Daly, Dr. Belinda Hatt, Dr. Kefeng Zhang, Dr. Peter Bach, Mr. Robert Sargent from Monash University, and Dr. Anja Randelovic from the University of Belgrade.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.02.193.

References

Allen, R.G., Pereira, L.S., Raes, D., Smith, M., 1998. Corp Evapotranspiration - Guidelines for Computing Crop Water Requirements - FAO Irrigation and Drainage Paper No.56. FAO - Food and Agriculture Organization of the United Nations, Rome, Italy.
Bais, H.P., Weir, T.L., Perry, L.G., Gilroy, S., Vivanco, J.M., 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. Annu. Rev. Plant Biol. 57, 233–266.
BOM, 2017. The Australian Government Bureau of Meteorology. http://www.bom.gov.au.
Bradford, S.A., Simunek, J., Walker, S.L., 2006. Transport and straining of *E. coli* O157:H7 in saturated porous media. Water Resour. Res. 42, W12S12.
Bratieres, K., Fletcher, T.D., Deletic, A., Zinger, Y., 2008. Nutrient and sediment removal by stormwater biofilters: a large-scale design optimisation study. Water Res. 42, 3930–3940.
Brauwere, A.D., Ouattara, N.K., Servais, P., 2014. Modeling fecal indicator bacteria concentrations in natural surface waters: a review. Crit. Rev. Environ. Sci. Technol. 44, 2380–2453.
Brown, J.S., Stein, E.D., Ackerman, D., Dorsey, J.H., Lyon, J., Carter, P.M., 2013. Metals and bacteria partitioning to various size particles in Ballona creek storm water runoff. Environ. Toxicol. Chem. 32, 320–328.
Burton, G.A., Pitt, R.E., 2002. Stormwater Effects Handbook: A Toolbox for Watershed Managers, Scientists and Engineers. Lewis Publishers, Boca Raton, Florida.
Caramia, M., Dell'Olmo, P., 2008. Multi-Objective Management in Freight Logistics - Increasing Capacity, Service Level and Safety with Optimization Algorithms. Springer, Verlag London.
Chandrasena, G.I., 2014. Faecal Microorganism Removal in Stormwater Biofilters. Department of Civil Engineering. Doctor of Philosophy. Monash University.
Chandrasena, G.I., Deletic, A., Lintern, A., Henry, R., McCarthy, D.T., 2017. Stormwater biofilters as barriers against *Campylobacter jejuni*, *Cryptosporidium* oocysts and adenoviruses; results from a laboratory trial. Water. 9, 949.
Chandrasena, G.I., Deletic, A., McCarthy, D.T., 2013. Evaluating *Escherichia coli* removal performance in stormwater biofilters: a preliminary modelling approach. Water Sci. Technol. 67, 2467–2475.
Chandrasena, G.I., Deletic, A., McCarthy, D.T., 2014. Survival of *Escherichia coli* in stormwater biofilters. Environ. Sci. Pollut. Res. 21, 391–401.
Chandrasena, G.I., Deletic, A., McCarthy, D.T., 2016. Biofiltration for stormwater harvesting: comparison of *Campylobacter spp.* and *Escherichia coli* removal under normal and challenging operational conditions. J. Hydrol. 537, 248–259.
Chandrasena, G.I., Pham, T., Payne, E.G., Deletic, A., McCarthy, D.T., 2014. *E. coli* removal in laboratory scale stormwater biofilters: influence of vegetation and submerged zone. J. Hydrol. 519, 814–822.
Cizek, A.R., Characklis, G.W., Krometis, L.-A., Hayes, J.A., Simmons III, O.D., Lonardo, S.D., et al., 2008. Comparing the partitioning behavior of *Giardia* and *Cryptosporidium* with that of indicator organisms in stormwater runoff. Water Res. 42, 4421–4438.
Crane, S.R., Moore, J.A., 1986. Modeling enteric bacterial die-off - a review. Water Air Soil Pollut. 27, 411–439.
Criss, R.E., Winston, W.E., 2008. Do Nash values have value? Discussion and alternate proposals. Hydrol. Process. 22, 2723–2725.
Daly, E., Zinger, Y., Deletic, A., Fletcher, T.D., 2009. A possible mechanism for soil moisture bimodality in humid-land environments. Geophys. Res. Lett. 36, L07402.
Dingman, S.L., 2002. Physical Hydrology. Upper Saddle River, N.J., Prentice Hall.
Dotto, C.B.S., Mannina, G., Kleidorfer, M., Vezzaro, L., Henrichs, M., McCarthy, D.T., et al., 2012. Comparison of different uncertainty techniques in urban stormwater quantity and quality modelling. Water Res. 46, 2545–2558.
Duncan, H.P., 1999. Urban Stormwater Quality: A Statistical Overview. Cooperative Research Centre for Catchment Hydrology, Melbourne, Australia.
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. Crit. Rev. Environ. Sci. Technol. 33, 299–361.
Fletcher, T.D., Deletic, A., Mitchell, V.G., Hatt, B., 2008. Reuse of urban runoff in Australia: a review of recent advances and remaining challenges. J. Environ. Qual. 37, 116–127.
Flint, K.P., 1987. The long-term survival of *Escherichia coli* in river water. J. Appl. Bacteriol. 63, 261–270.
Gargiulo, G., Bradford, S., Šimůnek, J., Ustohal, P., Vereecken, H., Klumpp, E., 2007. Bacteria transport and deposition under unsaturated conditions: the role of the matrix grain size and the bacteria surface protein. J. Contam. Hydrol. 92, 255–273.
Gargiulo, G., Bradford, S.A., Simunek, J., Ustohal, P., Vereecken, H., Klumpp, E., 2008. Bacteria transport and deposition under unsaturated flow conditions: the role of water content and bacteria surface hydrophobicity. Vadose Zone J. 7, 406–419.
Hathaway, J., Hhunt, W., Graves, A., Wright, J., 2011. Field evaluation of bioretention indicator bacteria sequestration in Wilmington, North Carolina. J. Environ. Eng. 137, 1103–1113.
Hatt, B.E., Fletcher, T.D., Deletic, A., 2007. Hydraulic and pollutant removal performance of stormwater filters under variable wetting and drying regimes. Water Sci. Technol. 56, 11–19.
IDEXX-Laboratories, 2007. Colilert® Test Kit, Edited. IDEXX-Laboratories, Maine, USA.
Le Coustumer, S., Fletcher, T.D., Deletic, A., Barraud, S., Poelsma, P., 2012. The influence of design parameters on clogging of stormwater biofilters: a large-scale column study. Water Res. 46, 6743–6752.
Li, Y.L., Deletic, A., Alcazar, L., Bratieres, K., Fletcher, T.D., McCarthy, D.T., 2012. Removal of *Clostridium perfringens*, *Escherichia coli* and F-RNA coliphages by stormwater biofilters. Ecol. Eng. 49, 137–145.
McCarthy, D.T., 2008. Modelling Microorganisms in Urban Stormwater. Department of Civil Engineering. Doctor of Philosophy. Monash University.
McCarthy, D.T., Deletic, A., Mitchell, V.G., Diaper, C., 2011. Development and testing of a model for micro-organism prediction in urban Stormwater (MOPUS). J. Hydrol. 409, 236–247.
McCarthy, D.T., Deletic, A., Mitchell, V.G., Fletchera, T.D., Diaper, C., 2008. Uncertainties in stormwater *E. coli* levels. Water Res. 42, 1812–1824.
Mourad, M., Bertrand-Krajewski, J.L., Chebbo, G., 2005. Calibration and validation of multiple regression models for stormwater quality prediction: data partitioning, effect of dataset size and characteristics. Water Sci. Technol. 52, 45–52.
Mukerji, K.G., Manoharachary, C., Singh, J., 2006. Microbial Activity in the Rhizosphere. Berlin Springer.
Nash, J.E., Sutcliffe, J.V., 1970. River flow forecasting through conceptual models part I - a discussion of principles. J. Hydrol. 10, 282–290.
NHMRC, 2008. Guidelines for Managing Risks in Recreational Water. Australian Government, National Health and Medical Research Council, Canberra, Australia.
Randelovic, A., Zhang, K., Jacimovic, N., McCarthy, D., Deletic, A., 2016. Stormwater biofilter treatment model (MPiRe) for selected micro-pollutants. Water Res. 89, 180–191.
Rusciano, G.M., Obropta, C.C., 2007. Bioretention column study: fecal coliform and total suspended solids reductions. Trans. ASABE 50, 1261–1269.
Schijven, J.F., Hassanizadeh, S.M., 2000. Removal of viruses by soil passage: overview of modeling, processes, and parameters. Crit. Rev. Environ. Sci. Technol. 30, 49–127.
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 Res. 38, 1355–1367.
Stott, R., Tondera, K., Blecken, G.T., Schreiber, C., 2017. Microbial Loads and Removal Efficiency Under Varying Flows. Ecotechnologies for the Treatment of Variable Stormwater and Wastewater Flows. Springer International Publishing, pp. 57–74.
Strehmel, N., Böttcher, C., Schmidt, S., Scheel, D., 2014. Profiling of secondary metabolites in root exudates of *Arabidopsis thaliana*. Phytochemistry 108, 35–46.
Tortora, G.J., Funke, B.R., Case, C.L., 1998. Microbiology: An Introduction. Addison-Wesley Publishing Company, Inc., California, USA.
Updegraff, D.M., 1983. Plugging and penetration of petroleum reservoir rock by microorganisms. 1982 International Conference on Microbial Enhancement of Oil Recovery. DOE, Bartlesville Energy Technology Cent (CONF-8205140), Afton, OK, USA, pp. 80–85.
Vezzaro, L., Mikkelsen, P.S., Deletic, A., McCarthy, D., 2013. Urban drainage models - simplifying uncertainty analysis for practitioners. Water Sci. Technol. 68, 2136–2143.
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 Environ. Res. 82, 701–714.
Zhang, L., Seagren, E.A., Davis, A.P., Karns, J.S., 2011. Long-term sustainability of *Escherichia coli* removal in conventional bioretention media. J. Environ. Eng. 137, 669–677.
Zhang, L., Seagren, E.A., Davis, A.P., Karns, J.S., 2012. Effects of temperature on bacterial transport and destruction in bioretention media: field and laboratory evaluations. Water Environ. Res. 84, 485–496.

3.3 Discussion and conclusions

The promising performance of the developed model when being tested with the data collected from various biofilter configurations demonstrated that the model could be broadly applied in different systems. In addition, the predictive model is adjustable under different operational conditions and weather patterns, such as various infiltration rates, inflow volumes and concentrations, and dry lengths between wet events. These results indicated that the model could reflect the effects of operational optimisation; e.g., how the system would perform if the infiltration rate or inflow volume is controlled. This is especially important for RTC modelling, as the major purpose of adopting RTC is to adjust the operational conditions to achieve system optimisation. Therefore, this predictive model for non-RTC biofilters has the potential to be employed for RTC strategy evaluation after modification.

However, only one set of laboratory experiments have been employed to test this model; how the model would perform in other biofilter systems (especially field systems) that with various design and operational conditions is still unknown. Moreover, whether model parameters that calibrated for one system are capable of predicting the performance of another system needs to be tested, to ensure the model could still provide reliable prediction when being applied in a system that no data are available (e.g., a newly developed system). More importantly, whether this model could be modified to fully reveal the benefits of RTC remains unseen, as no RTC data are available.

As such, several studies need be conducted: (1) test the model with additional data that collected from various systems, especially field systems, (2) analyse parameter transferability and prediction uncertainty of this model, (3) collect RTC data for model modification, and (4) modify the model and evaluate the modified model's performance in simulating biofilters that implemented with RTC strategies. All these studies will be presented and discussed in the following chapters.

3.4 References

Chandrasena G. I., Deletic A., McCarthy D. T. Evaluating *Escherichia coli* removal performance in stormwater biofilters: a preliminary modelling approach. Water Science & Technology 2013; 67: 2467-75.

Lund N. S. V., Falk A. K. V., Borup M., Madsen H., Mikkelsen P. S. Model predictive control of urban drainage systems: A review and perspective towards smart real-time water management. Critical Reviews in Environmental Science and Technology 2018; 48: 279-339.

Schütze M., Butler D., Beck B. M. Modelling, Simulation and Control of Urban Wastewater Systems: Springer Science & Business Media, 2002.

Zhang L., Seagren E. A., Davis A. P., Karns J. S. The Capture and Destruction of *Escherichia coli* from Simulated Urban Runoff Using Conventional Bioretention Media and Iron Oxide-coated Sand. Water Environment Research 2010; 82: 701-714.

Zhang L., Seagren E. A., Davis A. P., Karns J. S. Effects of temperature on bacterial transport and destruction in bioretention media: Field and laboratory evaluations. Water Environment Research 2012; 84: 485-496.

Chapter 4.

Model validation, parameter transferability analysis, and uncertainty analysis

4.1 Introduction

In Chapter 3, a predictive model for microbial removal in stormwater biofilters was developed. This model was tested using a set of laboratory experiments that explored the performance of various biofilter designs (i.e., different filter media and plant types). The model results indicated that the model prediction fitted the observation well for each of the tested biofilter configurations. Therefore, there is a potential to apply this model in aiding the design of biofilters in the future, and simulating real time control (RTC) for stormwater biofilters after modification.

However, how the model would perform when being applied in other biofilter systems that have different design features and operated under various conditions (especially the field systems that have larger scales and may experience extreme operational conditions) is still unknown, impeding a broader application of this model. More importantly, we need to analyse whether the model parameters that calibrated for one system are transferable to another system with similar design, to ensure that even if no data are available (e.g., for a newly developed system, or a large-scale field system where monitoring is very difficult to fulfil), the model could still provide reasonably good prediction by employing the parameter values that were calibrated for other systems. Although validation has been attempted by splitting the data into calibration part and validation part in Chapter 3, the model has not been successfully validated for all the tested biofilter configurations, as in some cases the model's performance during validation was never similar or better than what was observed during calibration. The main reason was proposed to be, 50% of the events were extreme in nature (e.g., of high volume and after long dry period), and thereby more data were required to adequately represent these events during calibration.

As such, the main objectives of this study are to test the performance of the developed model with additional datasets (other than the dataset used from model development) that were collected from biofilters with a wide range of designs and operational conditions, and analyse the transferability of the

calibrated parameters from one system to another (e.g., from a lab-scale system to a field-scale system). The research questions include:

- Is model capable of predicting microbial removal in various biofilter systems with different design and operational conditions?
- What is the prediction uncertainty when the parameters that calibrated with the data that collected from one biofilter system are transferred to another system with similar plant type and media type?

Corresponding to the research questions, two hypotheses were made:

- Since the governing processes and key operational factors for microbial removal have been included, this model is adequate to provide reasonably good microbial removal prediction in various biofilter systems;
- The plant type and media type are the most significant design factors that govern the microbial removal in stormwater biofilters; therefore, parameters that were calibrated with the data that collected from one biofilter system, can be transferred to another system with similar plant type and media type, with generally low prediction uncertainty.

This chapter presents the processes of model testing based on a set of laboratory-scale column experiments and a field system monitoring. The results of the sensitivity analysis are included in this chapter. In addition, parameter transferability analysis and prediction uncertainty analysis are also conducted, to comprehensively evaluate model's potential of being applied in various systems, especially the systems with limited data available (e.g., newly developed systems).

This chapter is written as a draft for journal publication and has been submitted to *Journal of Hydrology*.

4.2 Validation and uncertainty analysis of the developed model for microbial removal in stormwater biofilters

Validation and Uncertainty Analysis of a Stormwater Biofilter Treatment Model for Faecal Microorganisms

Pengfei Shen [a]*, David T. McCarthy [a], Gayani I. Chandrasena[b], Yali Li [a], Ana Deletic[c]

[a] Environmental and Public Health Microbiology Laboratory (EPHM Lab), Department of Civil Engineering, Monash University, Clayton, VIC 3800, Melbourne, Australia

[b] SMEC Australia, Level 5, Berry Street, North Sydney, NSW 2060

[c] School of Civil and Environmental Engineering, University of New South Wales, Sydney, NSW 2052, Australia

* Corresponding author. Email address: [redacted]

Abstract

Stormwater biofilters, also known as rain gardens or bioretention systems, are effective stormwater treatment systems. This paper presents the validation, sensitivity and uncertainty analyses of a model for microbial removal in stormwater biofilters. The model, previously developed based on a rather limited laboratory study, has been fully validated using the data collected in extensive laboratory experiments and field tests. The lab-scale and field-scale systems used for validation were of various designs (e.g., system size, plant type, media type, and the existence of submerged zone), and have been operated under a wide range of operational conditions (e.g., length of the antecedent dry period, and the inflow volume and concentration). For each tested biofilter design, the predicted *E. coli* concentrations in the biofilters' outflow showed relatively good agreement with the measured ones: e.g., Nash-Sutcliffe Efficiency (E_c) ranged from 0.50 to 0.60 for the laboratory tests, while E_c of 0.55 was obtained for the field system. The results from the sensitivity

analysis not only confirmed the significance of adsorption and desorption processes, but also revealed the impact of temperature on microbial die-off (which was not fully represented in the model development stage). Finally, parameter transferability from one system to another with similar design was examined, achieving generally promising E_c values (0.04 ~ 0.56 with the best-fit parameter set for the other system; maximum value: 0.46 ~ 0.63) and reasonable uncertainties (intersection percentage between prediction uncertainty band and observation with uncertainty intervals: 50 ~ 97 %). Most importantly, the prediction of *E. coli* outflow concentrations from the field system was reasonably good when laboratory-determined parameter values were adopted: with the best-fit parameter set for the lab-scale system, E_c = 0.39; maximum E_c = 0.55; intersection percentage between prediction and observation = 83 %. These results suggested that this rare biofilter model for microbial removal could provide reliable prediction for large-scale field systems, by simply calibrating parameters with limited laboratory-scale experiments.

Keywords

Stormwater biofilter; modelling; microbial removal; sensitivity analysis; model validation; *E. coli*

Introduction

Faecal microorganisms have been identified as a major pollutant in stormwater and reported to degrade waterways all over the world (Burton and Pitt, 2002; Ferguson et al., 2003). In addition, they impede stormwater harvesting due to the health risks they pose when contacting with humans (Fletcher et al., 2008).

To remove faecal microbes and other pollutants contained in stormwater, biofilters (also known as rain gardens or bioretention systems) have been widely applied in Australia and other countries. Stormwater biofilters are often effective for microbial removal: e.g., the microbial removal rates reported in a number of laboratory studies ranged from 73% to 99.9% (Chandrasena et al., 2014b; Li et al., 2012; Zhang et al., 2010; Zhang et al., 2011). However, in field studies, the microbial removal rate was found to be far more variable - it ranged

from 99% to even leaching (< 0) under challenging operational conditions (Chandrasena et al., 2016; Hathaway et al., 2011; Hathaway et al., 2009; Zinger et al., 2011). Unsurprisingly, the biofilter design impacts on microbial removal; the significance of filter media type, plant type and the existence of submerged zone were emphasized in a number of laboratory investigations (Chandrasena et al., 2014b; Li et al., 2016).

There are only a few models developed for the assessment of microbial removal by stormwater biofilters to aid the design of systems. These models range from very simple black box regressions to rather complex process-based models (Chandrasena et al., 2013; Shen et al., 2018b; Zhang et al., 2010; Zhang et al., 2012). Among them, the process-based model developed by Shen et al. (2018b) could be regarded as rather promising, as it showed good results in predicting *E. coli* concentrations in the outflow of five different biofilter designs tested in a controlled laboratory study (Chandrasena et al., 2017). This model, although process-based, is still rather simple; it contains a flow module and a microbial module that simulates adsorption, desorption, and die-off of microbes within stormwater biofilters.

However, before this model could be broadly adopted, its performance has to be tested on a wider range of biofiltration designs. Particularly, the model must be validated in field conditions, since field systems are often challenged due to unpredicted microbial inflows and extreme weather conditions (Daly et al., 2014; Göbel et al., 2007). In Australia, full-scale testing of stormwater harvesting systems is a must according to current guidelines (DHV, 2013); hence, any model that is to be used for designing of such systems must be fully validated in field conditions.

Furthermore, the uncertainty of any model outcomes needs to be assessed before a model is employed (Uusitalo et al., 2015). Since model parameters are often site-specific, accuracy and reliability of a model may be compromised when the parameters obtained from one system are transferred to another (Zhang et al., 2016). These are more likely to happen when the parameters calibrated at laboratory scale were used for field-scale predictions, as in the

field, uncertainties are more difficult to control than in a laboratory. However, adopting lab-based parameters for field predictions is sometimes the only option we have due to the large cost of field-scale monitoring programs (Zhang et al., 2016).

The aim of this study is to validate the process-based model developed by Shen et al. (2018b) for the prediction of microbial concentrations in stormwater biofilters' outflow. The main objectives include: (1) to test the model for the prediction of microbial concentrations under a wide range of biofilter designs and operational conditions, including the model testing on a full-scale field system; (2) to undertake global parameter sensitivity analysis to understand the significance of simulated microbial removal processes and the operational factor; (3) to analyse the transferability of the calibrated parameters from one system to another (including transferability of the calibrated parameters at laboratory scale to a field scale system); and (4) to assess prediction uncertainty in model prediction to understand the model's reliability. The presented work gave us confidence that the model can be applied to various biofiltration designs. The key finding was that the model calibrated in rather limited laboratory conditions could be used without further calibrations for large-scale field systems with reasonable accuracy. Since stormwater biofilters are often large systems that cannot be easily validated, the model could be used as a valuable tool for system design and evaluation.

Methods

2.1 Model description

The model fully explained in Shen et al. (2018b) consists of two modules: (1) water flow module, which describes the flow processes in biofilters; (2) microbial quality module, which simulates the microbial behaviours based on the results from water flow module. A "three-bucket" approach was applied in both modules; the buckets represent the three major parts of a typical biofilter: (a) ponding zone – a temporary pond on the top of filter media, (b) unsaturated zone – the unsaturated filter media, and (c) submerged zone – the saturated filter media created by a raised outflow pipe (Figure 1-left). For the biofilters without submerged zone (Figure 1-right), only the first two buckets were applied.

The water flow module has two parameters: (1) hydraulic conductivity, K_s, and (2) the plant coefficient for evapotranspiration, K_c, which reflects the water uptake ability of different plant species. In the microbial quality module, one-dimensional advection-dispersion equations were employed to simulate microbial transport and fate, with four parameters: adsorption rate (k_{att}), desorption rate (k_{det}), the standard die-off rate (μ_0) and the temperature correction coefficient for die-off (θ). The details of equations and parameters were fully presented in Shen et al. (2018b) with a summary in Supplementary material A.

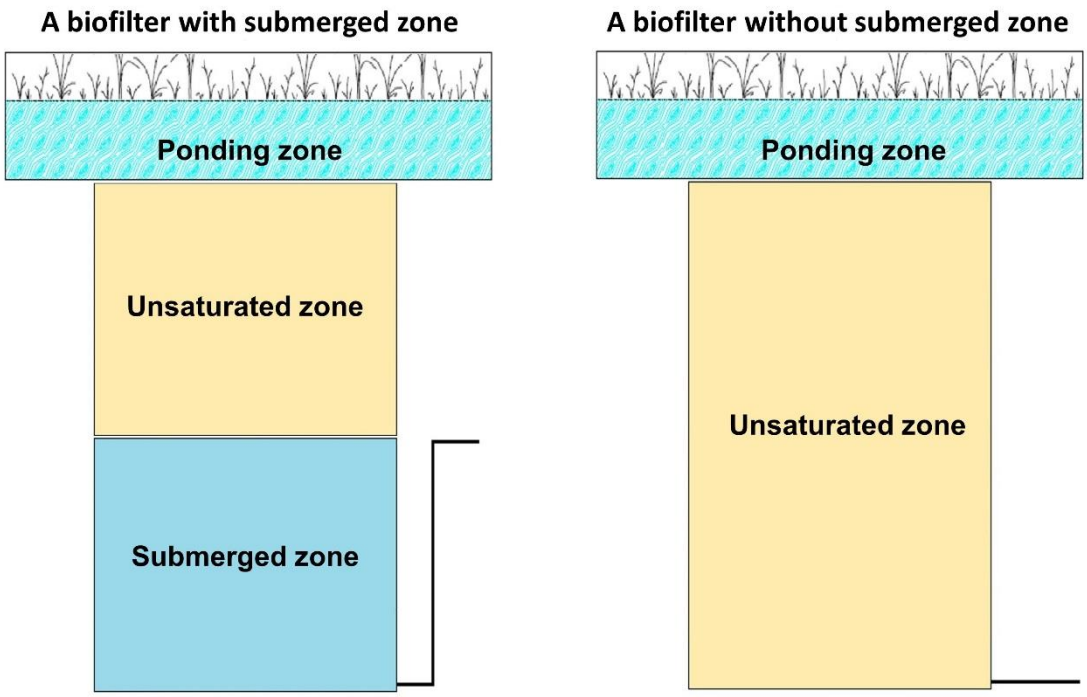


Figure 1 Schematic representations of stormwater biofilters with and without submerge zone (SZ) (adapted from Shen et al. (2018b)).

2.2 Validation data sets

The model was validated using two data sets: (1) the results of a number of laboratory experiments (different from the experimental data used for model development), and (2) the data collected from a field-scale system (Table 1). The laboratory experiments (named as *Lab* in Table 1) on three different biofilter designs (with 5 replications of each) were conducted in a greenhouse at Monash University in Australia and have been fully published in Li et al.

(2016). All of the biofilter designs had identical column size and filter media, but each design had different plant type (Table 1). The data collected from a field-scale biofilter located in the Royal Melbourne Golf Club, Cheltenham (a suburb of Melbourne, Australia) were also used (named as *Field* in Table 1). Experiments setup and data collection of this system have been published in Chandrasena et al. (2016).

Table 1 Design characteristics of biofilters in the two validation data sets. A Biofilter ID is given to each biofilter design according to its plant type: e.g., *Lab*-WS represents the unvegetated biofilters (washed sand only) in the *Lab* data set, *Field*-CP stands for the biofilters in *Field* data set that planted with *Carex appressa*.

Data set	**Plant**	**Biofilter ID**	**No. of replicates**	**Filter media**	**Biofilter size**
Lab (Li et al., 2016)	Unvegetated	*Lab*-WS	5	Washed sand, with a zeolite layer	Column diameter: 240 mm Ponding zone depth: 280 mm Unsaturated zone depth: 400 mm (zeolite layer depth: 150 mm) Submerged zone depth: 440 mm
	Palmetto buffalo	*Lab*-PB	5		
	Leptospermum continentale	*Lab*-LC	5		
Field (Chandrasena et al., 2016)	*Carex appressa*	*Field*-CP	N/A	Washed sand	Surface area: 860 m^2 Ponding zone depth: 300 mm Unsaturated zone depth: 950 mm No submerged zone

(1) Monitoring and sampling information of *Lab* data set

From 10/2012 to 08/2013, 42 dosing events were implemented (Table 2); each column was dosed with semi-natural stormwater (Bratieres et al., 2008; Li et al., 2016). Among them, 19 events were sampled and analysed. In non-sampling events, the dosing volume represents the average rainfall event size in Melbourne (equivalent loading: 5.75 mm); in sampling days, three challenging events with a dosing volume represents the 1 in 3 months average recurrence interval (ARI) (equivalent loading: 17.68 mm) were conducted, and in the other 16 sampling events the dosing volume represents 1 in 1 month ARI (equivalent loading: 8.84 mm). In each dosing event, *Escherichia coli* (*E. coli*) was spiked into inflow as a faecal microbial indicator, with a typical concentration found in stormwater runoff (Duncan, 1999) (Table 2). These events also have various lengths of antecedent dry days to represent the natural variability (Table 2). In

sampling days, inflow and outflow samples were collected; for outflow, the composited water remained in submerged zone from the previous event ("old" water), and composited newly treated outflow ("new" water) were respectively sampled. All the samples were transported on ice to the Environmental and Public Health Microbiology Laboratory (EPHM Lab) for enumeration of *E. coli* using the Colilert method™ (IDEXX-Laboratories, 2007). In each event, the instantaneous water depths in ponding zone after a regular interval and the total outflow volume were also recorded for each column.

(2) Monitoring and sampling information of *Field* data set

The stormwater runoff that collected from the catchment was pumped from temporary underground storage to the biofilters under a controlled inflow rate of 20 ~ 23 L/s (see Chandrasena et al. (2016) for full details). Electromagnetic flow meters were installed in both the inflow pipe and outflow pipe. Auto-samplers were utilised to collect inflow and outflow samples using a flow-weighted strategy: a 1 L water sample was taken after every 100,000 L water went through. From 05/2012 and 10/2013, 20 sampling events, with various event sizes, inflow concentrations and antecedent dry periods, were conducted (Table 2). All the collected samples were also transported on ice to EPHM Lab for *E. coli* analysis using the Colilert method™ (IDEXX-Laboratories, 2007). Due to data collection difficulty, the data of instantaneous water depths in ponding zone were not available.

Table 2 Sampling information of the two validation data sets: *Lab* and *Field*.

Data set	**Duration of monitoring**	**No. of sampling events**	**Antecedent dry days of sampling events**	**Inflow concentration of *E. coli***	**Data of instantaneous water depths in ponding zone**
Lab	11 months	19	1 day ~ 28 days	7.25 ~ 3.78×10^4 MPN/100mL	Available
Field	17 months	20	1 hour ~ 6.75 days	1×10^4 ~ 1.4×10^7 MPN/100mL	N/A

2.3 Calibration and sensitivity analysis

Water flow module and microbial quality module were calibrated separately. For each biofilter design, water flow module was calibrated firstly to obtain the flow conditions; afterwards, these flow conditions were used in the microbial quality

module for the prediction of microbial concentration. The Nash-Sutcliffe Efficiency, *E* (Nash and Sutcliffe, 1970), was used to evaluate the performance of each module.

(1) Calibration of water flow module

In the calibration of water flow module, each column in *Lab* data set was calibrated individually (i.e., replicate by replicate), as different operational conditions might inherently occur in different columns (e.g., clogging, cracks, and plant growth rates).

The instantaneous water depths in ponding zone were used to calibrate K_s (hydraulic conductivity), and the outflow volumes in each sampling event were used to calibrate K_c (plant coefficient for evapotranspiration). An equal-weighted multi-objective function of ponding water depths and event outflow volumes was employed to calculate the overall *E* of this module, E_q (Caramia and Dell'Olmo, 2008):

$$E_q = 0.5 \times (E_{depth} + E_{volume}) \qquad \text{Eq. (1)}$$

where E_{depth} is the *E* value calculated using the observed and predicted ponding water depths (for K_s), and E_{volume} is the *E* value calculated using the observed and predicted outflow volumes (for K_c).

For *Field*-CP, since no data were available for the calibration of water flow module, literature values of K_s and K_c were utilised to simulate water flow for the calibration of microbial quality module: Shen et al. (2018b) calibrated K_s and K_c for five biofilter columns that planted with *Carex appressa* and have washed sand as filter media; as *Field*-CP has the same plant type and media type, these two biofilter designs were assumed to have similar features regarding water flow; therefore, the mean values of calibrated K_s (197 mm/h) and K_c (1.2) in Shen et al. (2018b) were employed in this study.

(2) Calibration and sensitivity analysis of microbial quality module

In the calibration of microbial quality module, for each of the three biofilter designs in *Lab*, parameters were calibrated simultaneously for all five replicates,

as the differences of parameter values between replicates were assumed to be negligible, and individual column-by-column calibration was not supported by the water quality data.

Modified Monte-Carlo method that introduced by Vezzaro et al. (2013) was utilised for the calibration of *Lab* and *Field*. 100,000 parameter sets were randomly generated and tested in each biofilter design: k_{att} - adsorption rate (0.1 ~ 6 h^{-1}), μ_0 - standard die-off rate (1 ~ 4 day^{-1}), and θ - temperature correction coefficient for die-off (0.9 ~ 1.6) were uniformly distributed; k_{det} - desorption rate was log uniformly distributed (Log_{10} (k_{det}): -5 ~ 0.6 h^{-1}). The predicted *E. coli* concentrations in outflow by using each parameter set were recorded and compared with the observed data to calculate the *E* of this module, E_c; the parameter set that generated highest E_c was considered as the best-fit parameter set. All the *E. coli* concentration data were log-transformed, to represent the lognormal distribution of microbial concentrations (NHMRC, 2008), and to avoid bias (*E* favours peaks) (Criss and Winston, 2008). For each biofilter design, parameter distributions were generated by adopting the method introduced by Vezzaro et al. (2013) for ranking and selecting parameter sets; the parameter selection criterion was to ensure that the model prediction could cover ≥ 70 % of the observations, and if the number of parameter sets is further enlarged, only limited increment in observation coverage can be achieved. Parameter distributions of each configuration were plotted for sensitivity analysis.

For *Lab* data set, the model was run with a 1-minute time step (*dt*), and the space step (*dz*) for was set as 40 mm. For *Field* data set, the model was run with a 6-minute time step (*dt*), as only 6-minute rainfall data were available for *Field*, and the space step (*dz*) was set as 56 mm.

2.4 Parameter transferability and prediction uncertainty analyses

Parameter transferability and prediction uncertainty analyses were conducted between biofilters with similar filter media and plant, since media type and plant type are utmost crucial for microbial removal (Chandrasena et al., 2014b; Li et al., 2016). It is noted that, this study focused on the transferability of parameters

in microbial quality module (i.e., k_{att}, k_{det}, θ, and μ_0), as these parameters directly govern the microbial removal in biofilters.

In addition to the four biofilter designs in the two validation data sets (i.e., *Lab* and *Field*) (Table 1), the laboratory data set used to develop the model by Shen et al. (2018b) was also included. This data set (named *Lab-12Aug,* according to the system scale and the start date of the experiment) was collected by Chandrasena et al. (2017) from 08/2012 to 06/2013 on four biofilter designs that had similar filter media (washed sand) and plants (*Carex appressa*, *Palmetto buffalo*, *Leptospermum continentale*, and unvegetated) compared to the biofilters in *Field* and *Lab*. Each biofilter design in *Lab-12Aug* was also given a biofilter ID according to their plant type: e.g., *Lab-12Aug*-CP presents the biofilters planted with *Carex appressa* in *Lab-12Aug* data set (similar to the biofilter ID for *Field* and *Lab* in Table 1). Finally, the three data sets (*Lab*, *Field*, and *Lab-12Aug*) were divided into four groups according to the biofilter design, as shown Table 3 (each group had similar plant and media types).

Table 3 Four groups of biofilter designs for parameter transferability analysis. "Biofilter 1 using Biofilter 2" is short for "predicting Biofilter 1's *E. coli* concentrations using Biofilter 2's parameter sets" (e.g., "*Field*-CP using *Lab-12Aug*-CP" in Group CP stands for "predicting *Field*-CP's *E. coli* concentrations using *Lab-12Aug*-CP's parameter sets").

Group	**Biofilter ID**		**Parameter transferability and prediction uncertainty analyses**	
Group CP	*Field*-CP	*Lab-12Aug*-CP	*Field*-CP using *Lab-12Aug*-CP	*Lab-12Aug*-CP using *Field*-CP
Group WS	*Lab*-WS	*Lab-12Aug*-WS	*Lab*-WS using *Lab-12Aug*-WS	*Lab-12Aug*-WS using *Lab*-WS
Group PB	*Lab*-PB	*Lab-12Aug*-PB	*Lab*-PB using *Lab-12Aug*-PB	*Lab-12Aug*-PB using *Lab*-PB
Group LC	*Lab*-LC	*Lab-12Aug*-LC	*Lab*-LC using *Lab-12Aug*-LC	*Lab-12Aug*-LC using *Lab*-LC

The calibrated parameter sets for microbial removal module from one biofilter design were used to predict the *E. coli* concentrations of the other biofilter design that from the same group (while the water flow information of each biofilter was still obtained from individual calibration). For example, for Group CP (Table 3), the following steps were done: (i) *E. coli* concentrations were predicted in the outflows of *Field*-CP, with the calibrated parameter sets from *Lab-12Aug*-CP (*Field*-CP using *Lab-12Aug*-CP in Table 3); (ii) the Nash-

Sutcliffe Efficiency (E_c) of *Field*-CP prediction results with each parameter set from *Lab-12Aug*-CP were calculated; the E_c value when using the best-fit parameter set for *Lab-12Aug*-CP (i.e., using the parameter set that generated highest E_c value during the calibration of *Lab-12Aug*-CP), and the highest E_c value obtained in this step, were recorded; (iii) the 90 % uncertainty band of *Field*-CP prediction using *Lab-12Aug*-CP parameters was generated; the lower and upper bounds of the uncertainty band were respectively the 5th and 95th percentile of predicted *E .coli* concentrations (Beven and Binley, 1992); (iv) the observation data points with uncertainty intervals were printed with the prediction uncertainty band; the observation uncertainty intervals were ±30 % (as per McCarthy et al. (2008); including uncertainties occurred during sampling, sample transport, and analysis); (v) the intersections between the prediction uncertainty band and observation with uncertainty intervals were counted, and the intersection percentage was calculated: intersection percentage (%) = number of data points where intersection occurred / number of total data points; (vi) Steps (i) – (v) were repeated by adopting the parameter sets of *Field*-CP to predict outflow concentrations of *Lab-12Aug*-CP (i.e., *Lab-12Aug*-CP using *Field*-CP in Table 3). All these steps were implemented on each group in Table 3.

3. Results and discussion

3.1 Calibration

(1) Water flow module (*Lab* only)

Relatively high E_q was achieved for each replicate (ranging from 0.77 to 0.94), with 80% of E_q values being above 0.85 (Table 4). Due to the additional complexity introduced by plants, the vegetated biofilters (*Lab*-PB and *Lab*-LC) had lower E_q compared to unvegetated ones (*Lab*-WS). *Lab*-WS had higher K_s than *Lab*-PB and *Lab*-LC, as the existence of plant is more likely to cause clogging (Hatt et al., 2007); the K_c values of *Lab*-LC were higher than for the other two designs, as the large plant roots of *Leptospermum continentale* enhanced plant's transpiration capability (Le Coustumer et al., 2012). Slight differences in K_s and K_c values were found between replicates from a same design, due to the individual characteristics of each column (e.g., the plant growth status, and the occurrence of clogging).

Table 4 Calibrated K_s and K_c, and corresponding E_{depth}, E_{volume} and E_q for each column in *Lab*.

Biofilter design		Replicate1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
Lab-WS	K_s (mm/h)	239	228	222	235	202
	E_{depth}	0.92	0.85	0.83	0.87	0.83
	K_c (-)	1.2	0.9	0.9	1.1	1.1
	E_{volume}	0.97	0.98	0.97	0.93	0.97
	E_q	0.94	0.91	0.90	0.90	0.90
Lab-PB	K_s (mm/h)	128	116	91	113	89
	E_{depth}	0.88	0.88	0.84	0.87	0.85
	K_c (-)	1.0	1.6	0.6	1.0	1.0
	E_{volume}	0.94	0.83	0.94	0.94	0.92
	E_q	0.91	0.86	0.89	0.91	0.88
Lab-LC	K_s (mm/h)	85	94	95	95	81
	E_{depth}	0.82	0.82	0.85	0.81	0.84
	K_c (-)	1.6	1.6	1.5	1.8	1.6
	E_{volume}	0.74	0.73	0.87	0.97	0.74
	E_q	0.78	0.77	0.85	0.89	0.79

(2) Microbial quality module (*Lab* and *Field*)

Promising E_c values (0.50 ~ 0.60) were achieved for all tested biofilter designs (Table 5). Particularly, the model performance for the prediction of *Field* (E_c = 0.55) was as good as those for *Lab* (E_c = 0.50 ~ 0.60) (Table 5), despite that the field-collected data normally introduce more uncertainties compared to lab-collected data. Furthermore, this E_c value for *Field* was even higher than that achieved in a lab-scale system with similar plant type and media type (i.e., E_c = 0.46 for *Lab-12Aug*-CP, as per Shen et al. (2018b)), which was mainly due to the better data quality of *Field* data set (i.e., the data collected from *Field*-CP) compared to *Lab-12Aug*-CP (e.g., more sampling events and discrete outflow samples). Moreover, compared to other reported *E. coli* models, the E_c values achieved in this study were of high value: e.g., E_c ranged from 0.25 to 0.41 in a model developed for the prediction of microbial concentration in stormwater runoff (McCarthy et al., 2011). In addition, for each biofilter design, when the predicted outflow concentrations were plotted against observed ones, vast majority of data points were scattered around the 1:1 line and within the +/- one order of magnitude bar in Figure 2, reinforcing the finding that the prediction and observation were in good agreement for each biofilter design.

Table 5 Parameter values in the optimum calibrated parameter set for each biofilter design in *Field* and *Lab*, and their corresponding E_c. Parameter values reported in the literature are also provided for comparison.

	Biofilter ID	E_c	k_{att} (h^{-1})	Log (k_{det}) (h^{-1})	θ (-)	μ_0 (day^{-1})
Calibrated in this study	*Lab*-WS	0.54	2.82	-0.84	1.07	0.34
	Lab-PB	0.60	1.43	-1.41	1.13	0.43
	Lab-LC	0.50	1.79	-0.16	0.92	0.58
	Field-CP	0.55	1.41	-1.79	0.93	0.13
Reported in Shen et al. (2018b)	*Lab-12Aug*-CP	0.46	1.46	-3.12	1.14	0.64
	Lab-12Aug-WS	0.64	2.74	-0.35	1.07	0.61
	Lab-12Aug-PB	0.63	2.48	-0.04	1.13	1.11
	Lab-12Aug-LC	0.56	1.76	-0.53	1.01	0.03
Reported in other literature			0.20 ~ 5.86[a,b]	-4.22 ~ 0.31[a,b]	1.01 ~ 1.19[c]	0.06 ~ 1.23[d,e]

[a] Bradford et al. (2006); [b] Gargiulo et al. (2008); [c] Brauwere et al. (2014); [d] Chandrasena (2014); [e] Crane and Moore (1986)

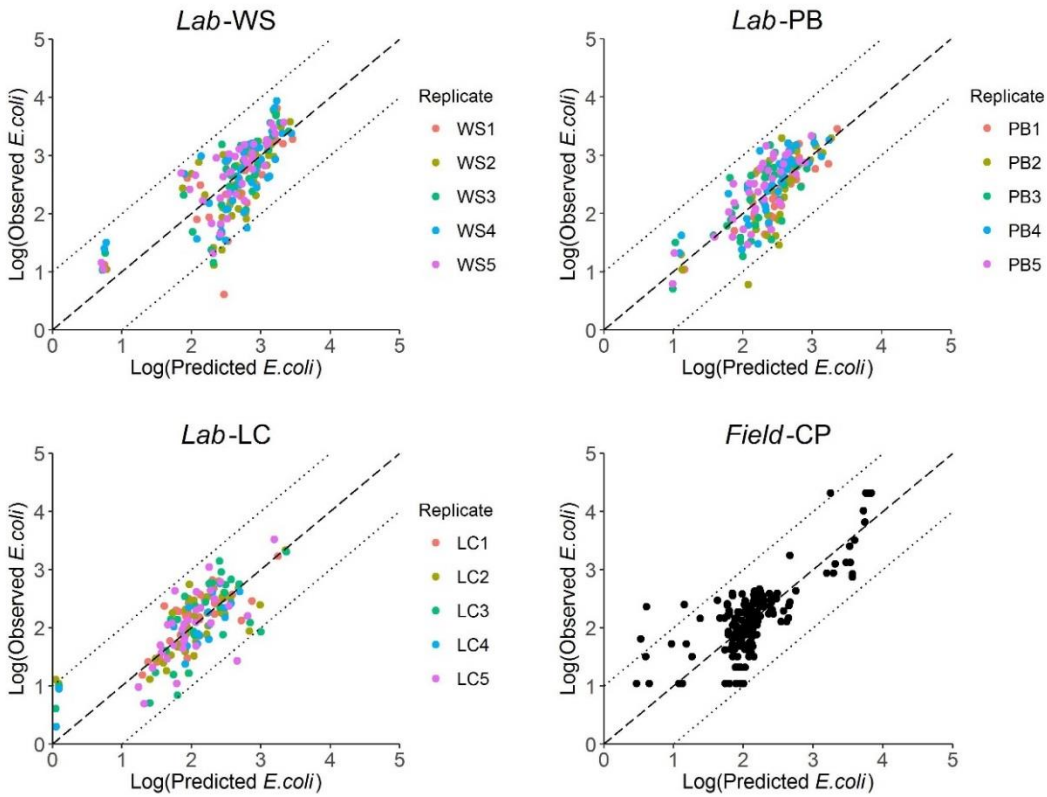


Figure 2 Comparison of observed and predicted outflow concentrations. Dashed lines indicate the 1:1 line between predicted and observed *E. coli* concentrations for *Lab* and *Field* data sets, while dotted lines indicate +/- one order of magnitude bars.

All the calibrated optimum parameter values were within the ranges reported in literature (Table 5). For example, the calibrated adsorption and desorption rates in this study felt within the ranges of the calibrated values for the same parameters when predicting microbial removal in saturated and unsaturated porous media (Bradford et al., 2006; Gargiulo et al., 2008). In particular, the calibrated die-off rates (μ_0) for *Lab* fitted those obtained from the measurements of laboratory biofilter columns very well: as per Chandrasena (2014), the measured die-off rates of columns plant with WS (unvegetated), PB (*Palmetto buffalo*), and LC (*Leptospermum continentale*) were 0.33 ~ 0.67 day^{-1}, 0.37 ~ 0.70 day^{-1}, and 0.32 ~ 0.77 day^{-1} respectively; all the calibrated values of *Lab*-WS, *Lab*-PB, and *Lab*-LC in this study were exactly within these ranges. Importantly, the calibrated μ_0 value of *Field*-CP (μ_0 = 0.13 day^{-1}) was lower than the measured values of laboratory columns that planted with *Carex appressa* (0.32 ~ 0.68 day^{-1}, as per Chandrasena (2014)). The hypothesised explanation is, the laboratory columns in Chandrasena (2014) had submerged zone but *Field*-CP was designed without submerged zone; since submerged zone could retain stormwater during dry days to experience longer time of die-off, and increase retention time during wet days by reducing hydraulic head for infiltration (Chandrasena et al., 2014b), the die-off rate of a system without submerged zone (e.g., *Field*-CP) are normally of low value compared to that of a system with submerged zone. However, the calibrated μ_0 value of *Field*-CP (μ_0 = 0.13 day^{-1}) was still within the ranges of reported die-off rates from other studies (e.g., 0.06 ~ 1.23 day^{-1} according to Crane and Moore (1986)).

In addition, compared to the findings in model development stage (as per Shen et al. (2018b)), the calibrated values of k_{att} for *Lab*-WS, *Lab*-LC and *Field*-CP, and the calibrated values of θ for *Lab*-WS and *Lab*-PB were almost identical to those reported for the systems with similar plant and media types. The differences in parameter values between this study and that study were mainly due to the differences in operational conditions (e.g., both *Lab* and *Field* data sets had more events than the study reported in Shen et al. (2018b); the growing status of plants would be various) and biofilter design (e.g., the existence of zeolite layer in *Lab*). The correlation between parameters during

calibration would also be a reason to cause differences in parameter values, which will be showed in sensitivity analysis in the next section.

It could be concluded that the model's performance in the prediction of *Lab* and *Field* biofilters were comparable to those reported in the model development phase by Shen et al. (2018b). This gives us confidence to use the model for a wide range of biofiltration designs and operational conditions.

3.2 Sensitivity analysis

Similar patterns of parameter distributions for each biofilter design in *Field* and *Lab* were obtained; therefore, only the results for *Field*-CP are presented here (Figure 3); the results for *Lab* biofilters are listed in Supplementary material B.

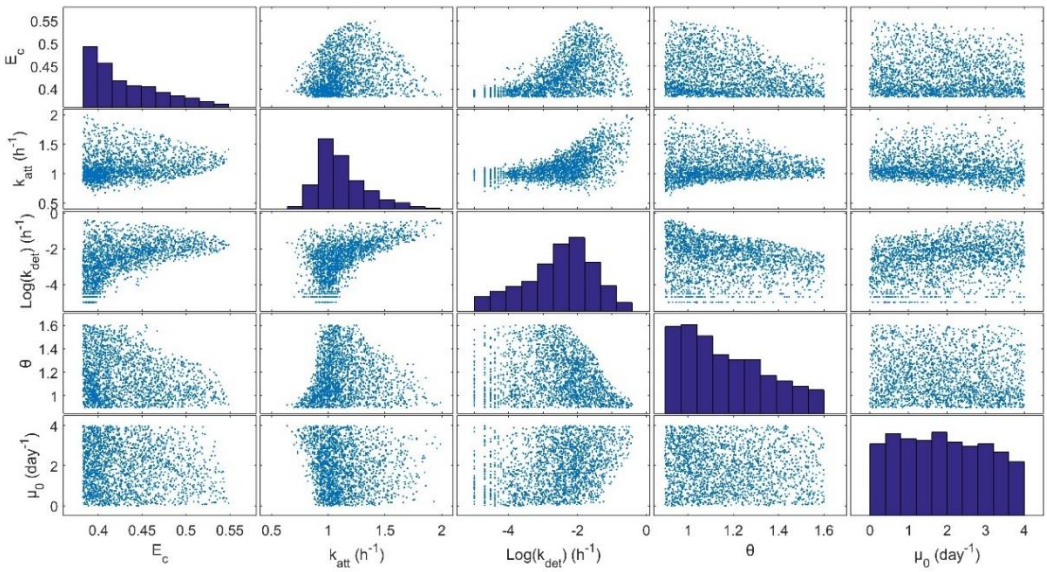


Figure 3 Matrix plot of *Field*-CP for sensitivity analysis. The diagonal histograms represent the distributions of E_c and all the parameters; the scatter plots between parameters reveal the parameter interactions.

For *Field*-CP, the peak in the diagonal histograms of k_{att}, k_{det} and θ in the matrix plot (Figure 3) suggested that the model was sensitive to these parameters (Dotto et al., 2012), indicating that adsorption and desorption processes were crucial for trapping microbes in *Field*-CP, and temperature is an influential factor for die-off.

The significance of k_{att} and k_{det} agreed with the findings in literature very well, as the majority of published studies reported that attachment and detachment were the most important processes for microbial removal (Bradford et al., 2006; Chandrasena et al., 2013; Stevik et al., 2004). In addition, temperature was also considered as a crucial factor for microbial survival (Chandrasena et al., 2014a; Stevik et al., 2004; Zhang et al., 2012), and the importance of it was confirmed in the sensitivity analysis in this study. It is noted that, in the model development stage, the importance of temperature was not successfully reflected, and the model was not very sensitive to the temperature coefficient (θ) in that study (Shen et al., 2018b). The improvement in this study was hypothesised due to the better data quality of *Field* compared that in Shen et al. (2018b) (e.g., more events and discrete outflow samples), and the temperature in the *Field* data set had higher fluctuation compared to the data set used for model development (which was collected from a greenhouse).

Importantly, the model is less sensitive to μ_0 than to other three parameters, although die-off rate governs the microbial removal during dry weather periods (Chandrasena et al., 2014a; Stevik et al., 2004; Zhang et al., 2012). In addition to the reason that parameter correlation and compensation occurred during calibration (Shen et al., 2018b), the importance of die-off could not be fully interpreted in *Field*-CP, as (1) *Field*-CP has no submerged zone, therefore, only limited water was retained in *Field*-CP to experience die-off during dry days; and (2) the dry period lengths in *Field* data set were not long enough to reflect die-off, as the longest dry period was only 6.75 days.

Similar results were found for *Lab* biofilters (Figure B.1, Figure B.2 and Figure B.3): the model was most sensitive to k_{att} and k_{det}; the sensitivity to θ was also enhanced compared to previous study in Shen et al. (2018b), due to a larger number of events in *Lab* data sets; the model is less sensitive to μ_0 , because (1) parameter correlations occurred, and (2) among 19 sampling events in *Lab*, only three of them have a dry period longer than 7 days, which might be not enough to reveal the importance of die-off (Shen et al., 2018b).

3.3 Parameter transferability and prediction uncertainty analyses

The E_c values obtained with the best-fit parameter set for the other system (e.g., for *Field*-CP by *Lab-12Aug*-CP, the E_c value obtained in *Field*-CP prediction with the best-fit parameter set for *Lab-12Aug*-CP) ranged from 0.04 to 0.56; these E_c values were favourable in comparison to other studies about parameter transferability analysis: e.g., Zhang et al. (2016) reported that when the best-fit parameters that estimated from laboratory experiments were used to predict micro-pollutants removal in field-scale biofilters, the E_c value could drop to -1.7. Acceptably high E_c values were achieved in most of the configurations (75% of E_c values were above 0.32), except the prediction of vegetated configurations in *Lab* (i.e., *Lab*-PB and *Lab*-LC) using the parameters from *Lab-12Aug*. The reason for the generally low E_c values in the prediction of *Lab*-PB and *Lab*-LC might be, compared to *Lab-12Aug*-PB and *Lab-12Aug*-LC, the soil moisture contents in these two configurations were probably influenced by the zeolite layer, and accordingly the plant growth status were different to those in *Lab-12Aug*-PB and *Lab-12Aug*-LC, which could dramatically impact microbial removal (Stevik et al., 2004).

In addition, all the maximum E_c values achieved in parameter transferability analysis (0.46 ~ 0.63 in Table 6) are similar to those in the calibration of *Lab*, *Field*, and *Lab-12Aug* biofilters (0.46 ~ 0.64 in Table 5). All these results indicated that the model performance in microbial removal prediction was not obviously diminished when adopting the calibrated parameter values from another system with similar plant and media types.

Especially, when the lab-based parameters (calibrated from *Lab-12Aug*-CP) for both water flow module and microbial quality module were used to predict the concentrations in discrete outflow samples from a field-scale system (*Field*-CP), an promising E_c value (0.39) was achieved when the best-fit parameter set for *Lab-12Aug*-CP was adopted (Table 6), and the maximum E_c value (0.55) was exactly same to that in *Field*-CP calibration (Table 5).

Table 6 E_c of *E. coli* predictions in parameter transferability analysis, and the Intersection percentage (%) between the prediction uncertainty bands and observation with uncertainty intervals for each configuration. The form of E_c values is: E_c value obtained with the best-fit parameter set for the other system (the maximum E_c value achieved in the prediction with all the calibrated parameter sets for the other system).

E_c of *E. coli* predictions in parameter transferability analysis		**Intersection percentage (%) between the prediction uncertainty bands and observation with uncertainty intervals**	
Lab-12Aug-CP using *Field*-CP 0.32 (0.46)	*Field*-CP using *Lab-12Aug*-CP 0.39 (0.55)	*Lab-12Aug*-CP using *Field*-CP 50 %	*Field*-CP using *Lab-12Aug*-CP 83 %
Lab-12Aug-WS using *Lab*-WS 0.56 (0.58)	*Lab*-WS using *Lab-12Aug*-WS 0.45 (0.54)	*Lab-12Aug*-WS using *Lab*-WS 97 %	*Lab*-WS using *Lab-12Aug*-WS 71 %
Lab-12Aug-PB using *Lab*-PB 0.44 (0.63)	*Lab*-PB using *Lab-12Aug*-PB 0.04 (0.60)	*Lab-12Aug*-PB using *Lab*-PB 73 %	*Lab*-PB using *Lab-12Aug*-PB 81 %
Lab-12Aug-LC using *Lab*-LC 0.36 (0.56)	*Lab*-LC using *Lab-12Aug*-LC 0.05 (0.48)	*Lab-12Aug*-LC using *Lab*-LC 59 %	*Lab*-LC using *Lab-12Aug*-LC 71 %

The intersection percentages between the prediction uncertainty bands and observation with uncertainty intervals (Table 6) further substantiated that the model prediction in parameter transferability analysis was reliable: all the intersection percentages were above 50 %; for the prediction of *Field*-CP using *Lab-12Aug*-CP parameters, 83 % of observed outflow concentrations with uncertainty intervals intersected with the prediction uncertainty band, demonstrating the outstanding performance of the model when it was only calibrated with lab-based data for field-scale system prediction; for parameter transferability analyses between *Lab* and *Lab-12Aug* biofilters, the intersection percentage ranged from 59 % to 97 %; the lowest intersection percentage (50 %) was achieved when *Lab-12Aug*-CP was predicted with *Field*-CP parameters. Compared to other studies about stormwater pollutants removal, these percentages could be considered as of high values: for example, Dotto et al. (2012) used four different techniques in urban drainage modelling to analyse the uncertainty in total suspended solids removal prediction, only 22.8 ~ 28.8 % observations were intersected with the 90% uncertainty band.

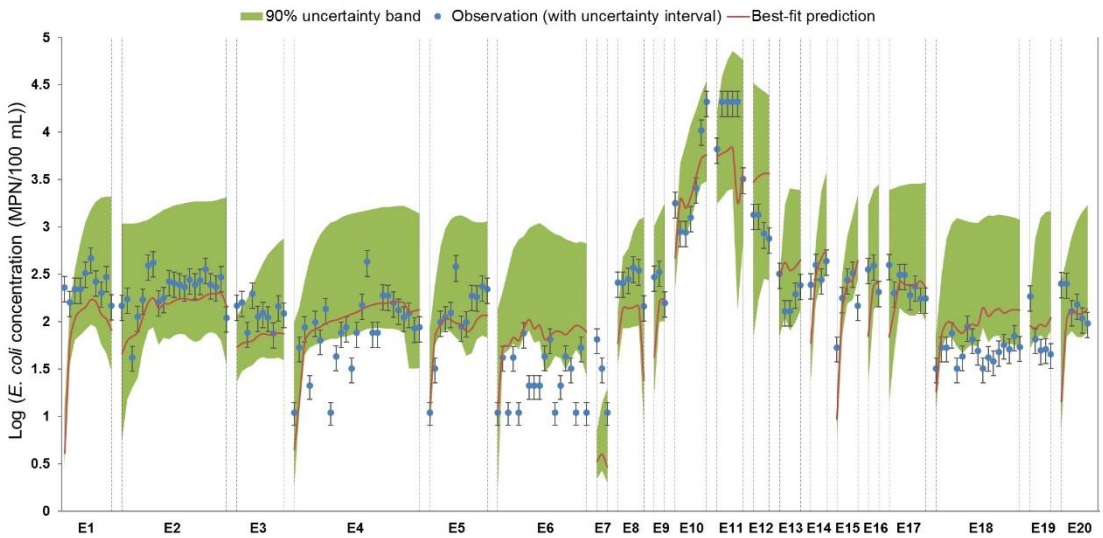


Figure 4 Uncertainty analysis of *E. coli* removal prediction for *Field*-CP using *Lab-12Aug*-CP parameters, including observation with uncertainty intervals, the best-fit prediction and 90% uncertainty band. E*n* stands for Event *n* (the *n*th event).

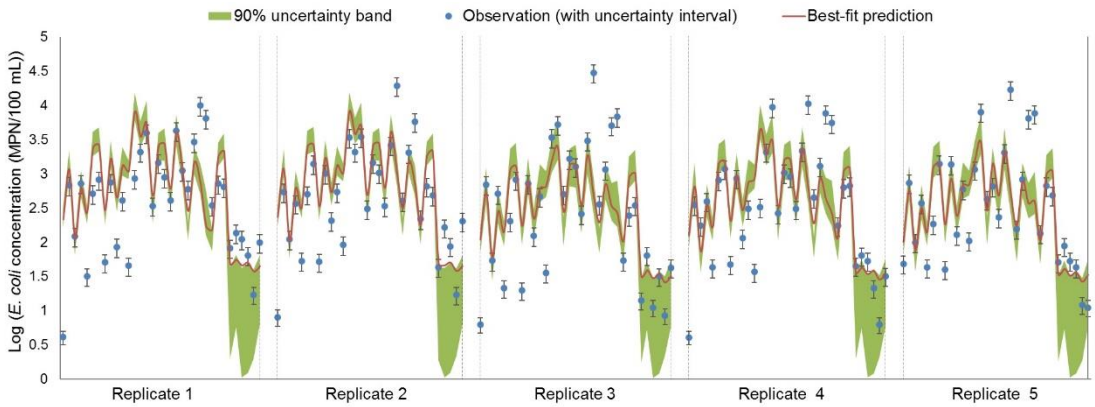


Figure 5 Uncertainty analysis of *E. coli* removal prediction for *Lab-12Aug*-CP using *Field*-CP parameters, including observation with uncertainty intervals, the best-fit prediction and 90% uncertainty band.

For the prediction of *Field*-CP using *Lab-12Aug*-CP parameters, the outliers of intersection (i.e., where intersection between the prediction uncertainty band and observation with uncertainty intervals did not occur) were mainly the data points from the events with extremely high inflow volumes (Figure 4). That was because, the inflow volumes in some events (e.g., Event 6 and Event 18) of *Field*-CP could reach to 10 pore volumes of the biofilter; however, the maximum inflow volume for *Lab-12Aug*-CP was only two pore volumes. Therefore, the

parameter calibrated from *Lab-12Aug*-CP data did not capture the features regarding microbial removal during high-volume events; accordingly, the prediction of *Field*-CP for these events is not as accurate as for other events.

For the prediction of *Lab-12Aug*-CP using *Field*-CP parameters, the comparatively low percentage (50 %) of intersection between observation and prediction (Figure 5) is due to the poor prediction of “old water” (submerged zone water retained from the previous event) in *Lab-12Aug* samples for most events. That was because, (1) for *Field*-CP, the longest dry period was 6.75 days, which was shorter than 25% percent of the dry periods of *Lab-12Aug*-CP (Chandrasena et al., 2017); (2) *Lab-12Aug*-CP columns had submerged zone (*Field*-CP does not), which performed as an extra site for microbes to experience die-off during dry days. These two major differences hindered *Field-CP* from fully representing the die-off process in *Lab-12Aug*-CP biofilters during dry days, especially in dry days with a length longer than 6.75 days; accordingly, high uncertainty occurred in predicting the “old water” samples that collected in events after long dry days in *Lab-12Aug*-CP with *Field*-CP parameters.

For *Lab* and *Lab-12Aug* biofilters, the uncertainty analyses results including the prediction uncertainty bands using the other system’s parameter sets and observations with uncertainty intervals could be found in Supplementary material B (Figure B.4 ~ Figure B.9). High intersection percentages (59 % to 97 %) were found, despite *Lab* and *Lab-12Aug*’s slight differences in media type (*Lab* biofilters include a zeolite layer but *Lab-12Aug* biofilters do not) and operational conditions (e.g., *Lab-12Aug* included three events with restricted flow; *Lab* had two more flushing events than *Lab-12Aug*), reinforcing the transferability of the model parameters. In addition, the averaged intersection percentages for each plant type followed the order of: unvegetated biofilters (84 % for WS) > *Palmetto buffalo* biofilters (77 % for PB) > *Leptospermum continentale* biofilters (65 % for LC), as some uncertainties introduced and/or enhanced by plants (e.g., preferential flow) could be not captured by the model, and the plants with denser roots are more inclined to lead to these uncertainties (roots density: *Leptospermum continentale* > *Palmetto buffalo*).

4. Conclusion

A recently developed stormwater biofilter treatment model for faecal microorganisms was successfully validated with the independently collected data sets from lab-scale and field-scale biofilters with various designs and operational schemes, demonstrating the broad application of this model.

The sensitivity analyse reinforced the significance of adsorption and desorption. In addition, the importance of temperature to die-off, which was not fully represented in the previous study, was also revealed. This finding indicates that better data quality could better reflect the importance of parameters.

Most importantly, a promising parameter transferability of this model was found, as good agreement was found between prediction and observation, even when the parameters from another system with similar plant type and media type were adopted. The results of uncertainty analysis further demonstrated the reliability of model prediction in parameter transferability analysis, as a generally low uncertainty was obtained in the prediction of each configuration.

It was also concluded that the model could be applied to large-scale field systems after being calibrated with only simple laboratory tests, as a good agreement between prediction and observation with low prediction uncertainty was achieved when the parameters calibrated entirely from a laboratory study were employed to predict a field system. Considering that stormwater biofilters are often large systems that cannot be easily validated, the model could be widely utilised as a valuable tool for system design and evaluation.

Acknowledgement

The authors would like to thank the Royal Melbourne Golf Club for the assistance in field monitoring, and CRC for Water Sensitive Cites for the help in laboratory data collection.

References

Beven, K., Binley, A., 1992. The future of distributed models: Model calibration and uncertainty prediction. Hydrol. Process., 6: 279-298

Bradford, S.A., Simunek, J., Walker, S.L., 2006. Transport and straining of *E. coli* O157:H7 in saturated porous media. Water Resour. Res., 42(12): W12S12

Bratieres, K., Fletcher, T.D., Deletic, A., Zinger, Y., 2008. Nutrient and sediment removal by stormwater biofilters: A large-scale design optimisation study. Water Res., 42: 3930-3940

Brauwere, A.d., Ouattara, N.K., Servais, P., 2014. Modeling Fecal Indicator Bacteria Concentrations in Natural Surface Waters: A Review. Crit. Rev. Environ. Sci. Technol., 44: 2380-2453

Burton, G.A., Pitt, R.E., 2002. Stormwater effects handbook: a toolbox for watershed managers, scientists and engineers. Lewis Publishers, Boca Raton, Florida

Caramia, M., Dell'Olmo, P., 2008. Multi-objective Management in Freight Logistics - Increasing Capacity, Service Level and Safety with Optimization Algorithms. Springer-Verlag London

Chandrasena, G.I., 2014. Faecal Microorganism Removal in Stormwater Biofilters, Monash University

Chandrasena, G.I., Deletic, A., A., L., Henry, R., McCarthy, D.T., 2017. Stormwater biofilters as barriers against *Campylobacter jejuni*, *Cryptosporidium* oocysts and adenoviruses; results from a laboratory trial. Water, 9: 949

Chandrasena, G.I., Deletic, A., McCarthy, D.T., 2013. Evaluating *Escherichia coli* removal performance in stormwater biofilters: a preliminary modelling approach. Water Sci. Technol., 67(11): 2467-75

Chandrasena, G.I., Deletic, A., McCarthy, D.T., 2014a. Survival of *Escherichia coli* in stormwater biofilters. Environ. Sci. Pollut. Res., 21(8): 391-401

Chandrasena, G.I., Deletic, A., McCarthy, D.T., 2016. Biofiltration for stormwater harvesting: Comparison of *Campylobacter spp.* and *Escherichia coli* removal under normal and challenging operational conditions. J. Hydrol., 537: 248-259

Chandrasena, G.I., Pham, T., Payne, E.G., Deletic, A., McCarthy, D.T., 2014b. *E. coli* removal in laboratory scale stormwater biofilters: Influence of vegetation and submerged zone. J. Hydrol., 519: 814-822

Crane, S.R., Moore, J.A., 1986. Modeling Enteric Bacterial Die-off - A Review. Water, Air, Soil Pollut., 27: 411-439

Criss, R.E., Winston, W.E., 2008. Do Nash values have value? Discussion and alternate proposals. Hydrol. Process., 22: 2723-2725

Daly, E., Bach, P.M., Deletic, A., 2014. Stormwater pollutant runoff: a stochastic approach. AdWR, 74: 148-155

DHV, 2013. Guidelines for validating treatment processes for pathogen reduction: Supporting Class A recycled water schemes in Victoria. Department of Health, Victoria

Dotto, C.B.S. et al., 2012. Comparison of different uncertainty techniques in urban stormwater quantity and quality modelling. Water Res., 46: 2545-2558

Duncan, H.P., 1999. Urban Stormwater Quality: A Statistical Overview. Cooperative Research Centre for Catchment Hydrology, Melbourne, Australia

Ferguson, C., De Roda Husman, A.M., Altavilla, N., Deere, D., Ashbolt, N., 2003. Fate and transport of surface water pathogens in watersheds. Crit. Rev. Environ. Sci. Technol., 33: 299-361

Fletcher, T.D., Deletic, A., Mitchell, V.G., Hatt, B., 2008. Reuse of urban runoff in Australia: a review of recent advances and remaining challenges. J. Environ. Qual., 37(5 Supplement): 116 - 127

Gargiulo, G. et al., 2008. Bacteria Transport and Deposition under Unsaturated Flow Conditions: The Role of Water Content and Bacteria Surface Hydrophobicity. Vadose Zone J., 7: 406-419

Göbel, P., Dierkes, C., Coldewey, W.G., 2007. Storm water runoff concentration matrix for urban areas. J. Contam. Hydrol., 91: 26-42

Hathaway, J., Hhunt, W., Graves, A., Wright, J., 2011. Field Evaluation of Bioretention Indicator Bacteria Sequestration in Wilmington, North Carolina. J. Environ. Eng., 137: 1103-1113

Hathaway, J.M., Hunt, W.F., Jadlocki, S., 2009. Indicator bacteria removal in storm-water best management practices in charlotte, north carolina. J. Environ. Eng., 135: 1275-1285

Hatt, B.E., Fletcher, T.D., Deletic, A., 2007. Hydraulic and pollutant removal performance of stormwater filters under variable wetting and drying regimes. Water Sci. Technol., 56(12): 11-19

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

Le Coustumer, S., Fletcher, T.D., Deletic, A., Barraud, S., Poelsma, P., 2012. The influence of design parameters on clogging of stormwater biofilters: a large-scale column study. Water Res., 46(20): 6743-52

Li, Y., McCarthy, D.T., Deletic, A., 2016. *Escherichia coli* removal in copper-zeolite-integrated stormwater biofilters: Effect of vegetation, operational time, intermittent drying weather. Ecol. Eng., 90: 234-243

Li, Y.L. et al., 2012. Removal of Clostridium perfringens, Escherichia coli and F-RNA coliphages by stormwater biofilters. Ecol. Eng., 49: 137-145

McCarthy, D.T., Deletic, A., Mitchell, V.G., Diaper, C., 2011. Development and testing of a model for Micro-Organism Prediction in Urban Stormwater (MOPUS). J. Hydrol., 409(1-2): 236-247

McCarthy, D.T., Deletic, A., Mitchell, V.G., Fletchera, T.D., Diaper, C., 2008. Uncertainties in stormwater E. coli levels. Water Res., 42: 1812-1824

NHMRC, 2008. Guidelines for Managing Risks in Recreational Water. Australian Government, National Health and Medical Research Council, Canberra, Australia

Shen, P., Deletic, A., Urich, C., Chandrasena, G.I., McCarthy, D.T., 2018. Stormwater Biofilter Treatment Model for Faecal Microorganisms. Sci. Total Environ., 630: 992-1002

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 Res., 38(6): 1355-1367

Uusitalo, L., Lehikoinen, A., Helle, I., Myrberga, K., 2015. An overview of methods to evaluate uncertainty of deterministic models in decision support. Environ. Model. Software, 63: 24-31

Vezzaro, L., Mikkelsen, P.S., Deletic, A., McCarthy, D., 2013. Urban drainage models - simplifying uncertainty analysis for practitioners. Water Sci. Technol., 68(10): 2136-43

Zhang, K., Randelovic, A., Deletic, A., Page, D., T.McCarthy, D., 2016. Stormwater biofilters: A new validation modelling tool. Ecol. Eng., 87(53-61)

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 Environ. Res, 82(8): 701-714

Zhang, L., Seagren, E.A., Davis, A.P., Karns, J.S., 2011. Long-term sustainability of escherichia coli removal in conventional bioretention media. J. Environ. Eng., 137: 669-677

Zhang, L., Seagren, E.A., Davis, A.P., Karns, J.S., 2012. Effects of temperature on bacterial transport and destruction in bioretention media: Field and laboratory evaluations. Water Environ. Res, 84: 485-496

Zinger, Y., Deletic, A., Fletcher, T.D., Breen, P., Wong, T., 2011. A Dual-mode Biofilter System: Case study in Kfar Sava, Israel, 12th International Conference on Urban Drainage, Porto Alegre, Brazil

Supplementary material A. Full details of model description and equations

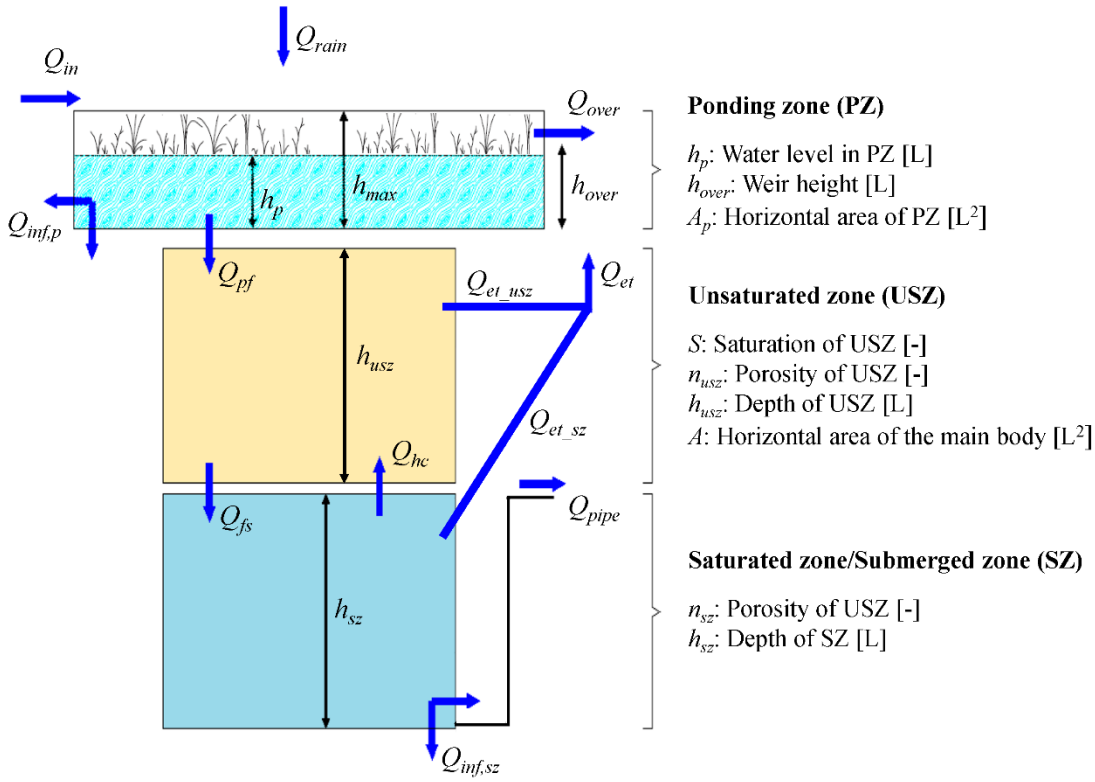


Figure A.1 Schematic representation of a typical stormwater biofilter and its flow scheme, and the key variables in each part. Stormwater inflow (Q_{in}), rainfall precipitation (Q_{rain}), overflow (Q_{over}), water flow from PZ to USZ (Q_{pf}), flow from the USZ to SZ (Q_{fs}), evapotranspiration (Q_{et}; divided to the evapotranspiration from USZ (Q_{et_usz}) and from SZ (Q_{et_sz})), capillary rise (Q_{hc}), infiltration into the surrounding soil ($Q_{inf,p}$ and $Q_{inf,sz}$) and outflow (Q_{pipe}).

Table A.1 Equations for the water flow module.

Water flow module equation	**Eq. No.**
General form of equations	
Flow = min (physically possible; available upstream; available downstream)	
Ponding zone (PZ)	
Infiltration from PZ to unsaturated zone	
$Q_{pf} = \min\left(K_s A \frac{h_p + h_{usz}}{h_{usz}}, \frac{h_p A_p}{dt} + Q_{in} + Q_{rain}, \frac{1}{dt}(1-S) n_{usz} h_{usz} A\right)$	(A.1)
Infiltration from PZ to the surrounding soil	
$Q_{inf,p} = \begin{cases} \min\left(K_f\left[(A_p - A) + C_s h_p P_p\right], \frac{h_p A_p}{dt}\right), \text{if unlined} \\ 0, \text{if lined} \end{cases}$	(A.2)
Overflow through weirs	
$Q_{over} = \begin{cases} \min\left(C_Q B \sqrt{2g(h_p - h_{over})^3}, \frac{A_p(h_p - h_{over})}{dt}\right), h_p > h_{over} \\ 0, h_p \le h_{over} \end{cases}$	(A.3)
Water mass balance in PZ	
$\frac{d(h_p A_p)}{dt} = Q_{in} + Q_{rain} - Q_{pf} - Q_{over} - Q_{inf,p}$	(A.4)
Unsaturated zone and saturated zone (USZ and SZ)	
Entire Saturation in USZ plus SZ	
$S_{entire} = \frac{S \times n_{usz} h_{usz} + n_{sz} h_{sz}}{n_{usz} h_{usz} + n_{sz} h_{sz}}$	(A.5)
Total Evapotranspiration from USZ and SZ	
$Q_{et} = \begin{cases} 0, S_{entire} \le S_w \\ A \times K_c \times ET_0 \frac{S_{entire} - S_w}{S_s - S_w}, S_w < S_{entire} \le S_s \\ A \times K_c \times ET_0, S_s < S_{entire} \le 1 \end{cases}$	(A.6)
Unsaturated zone (USZ)	
Evapotranspiration from USZ	
$Q_{et\_usz} = Q_{et} \times \frac{S \times n_{usz} h_{usz}}{S \times n_{usz} h_{usz} + n_{sz} h_{sz}}$	(A.7)
Flow due to capillary rise	
$Q_{hc} = A C_r (S - S_s)(S_{fc} - S), C_r = \frac{4 \times K_c \times ET_0}{2.5(S_{fc} - S_s)^2}$	(A.8)
when $S_s \le S \le S_{fc}$, otherwise $Q_{hc} = 0$	
Infiltration from USZ to SZ	
$Q_{fs} = \begin{cases} \min\left(A \times K_s \frac{h_p + h_{usz}}{h_{usz}} S^{\gamma}, \frac{(S - S_{fc}) A \times n_{usz} h_{usz}}{dt} + Q_{pf} + Q_{hc}\right), S \ge S_{fc} \\ 0, S < S_{fc} \end{cases}$	(A.9)
Water mass balance in USZ	
$\frac{d(S \times n_{usz} h_{usz} A)}{dt} = Q_{pf} + Q_{hc} - Q_{fs} - Q_{et\_usz}$	(A.10)
Saturated zone (SZ)	
Evapotranspiration from SZ	
$Q_{et\_sz} = Q_{et} \times \frac{n_{sz} h_{sz}}{S \times n_{usz} h_{usz} + n_{sz} h_{sz}} = Q_{et} - Q_{et\_usz}$	(A.11)
Infiltration from SZ to the surrounding soil	
$Q_{inf,sz} = \begin{cases} \min\left(K_f (A + C_s P_{sz} h_{sz}), \frac{n_{sz} h_{sz} A}{dt}\right), \text{if unlined} \\ 0, \text{if lined} \end{cases}$	(A.12)
Flow through drainage pipe	
$Q_{pipe} = \begin{cases} \min\left(A \times K_s \frac{h_p + h_{usz}}{h_{usz} + h_{sz}}, \frac{(h_{sz} - h_{pipe}) n_{sz} A}{dt} + Q_{fs} - Q_{hc} - Q_{et\_sz} - Q_{inf,sz}\right), h_{sz} > h_{pipe} \\ 0, h_{sz} \le h_{pipe} \end{cases}$	(A.13)

Table A.2 Equations for the microbial quality module.

Microbial quality module equation	**Eq. No.**
Ponding zone (PZ)	
Microbial mass balance in PZ	
$\frac{d(c_p h_p A_p)}{dt} = c_{in} Q_{in} - c_p(Q_{pf} + Q_{over} + Q_{inf,p}) - \mu c_p h_p A_p$	(A.14)
Unsaturated zone (USZ)	
Microbial mass balance in the water phase	
$\frac{\partial(Sn_{usz}c_{usz})}{\partial t} + (Sn_{usz}k_{att}c_{usz} - \rho k_{det}M_1) = \frac{\partial}{\partial z}\left(Sn_{usz}D_1\frac{\partial c_{usz}}{\partial z}\right) - \frac{\partial(q_1 c_{usz})}{\partial z} - Sn_{usz}\mu c_{usz}$	(A.15)
Adsorption, desorption and die-off of adsorbed microbes in the soil phase	
$\frac{\partial M_1}{\partial t} = \frac{n_{usz}S}{\rho}k_{att}c_{usz} - k_{det}M_1 - \mu M_1$	(A.16)
Dispersion coefficient in USZ	
$D_1 = \lambda\frac{q_1}{n_{usz}S}$	(A.17)
Average unit flow in USZ	
$q_1 = \frac{\alpha_1(Q_{pf} - Q_{et}) + \beta_1(Q_{fs} - Q_{hc})}{A}$	(A.18)
where $\alpha_1 + \beta_1 = 1$, and $\alpha_1 = 1$ at upper boundary, $\beta_1 = 1$ at lower boundary	
Saturated zone (SZ)	
Microbial mass balance in the water phase	
$\frac{\partial(n_{sz}c_{sz})}{\partial t} + (n_{sz}k_{att}c_{sz} - \rho k_{det}M_2) = \frac{\partial}{\partial z}\left(n_{sz}D_2\frac{\partial c_{sz}}{\partial z}\right) - \frac{\partial(q_2 c_{sz})}{\partial z} - n_{sz}\mu c_{sz}$	(A.19)
Adsorption, desorption and die-off of adsorbed microbes in the soil phase	
$\frac{\partial M_2}{\partial t} = \frac{n_{sz}}{\rho}k_{att}c_{sz} - k_{det}M_2 - \mu M_2$	(A.20)
Dispersion coefficient in SZ	
$D_2 = \lambda\frac{q_2}{n_{sz}}$	(A.21)
Average unit flow in SZ	
$q_2 = \frac{\alpha_2(Q_{fs} - Q_{hc}) + \beta_2(Q_{pipe} + Q_{inf,sz})}{A}$	(A.22)
where $\alpha_2 + \beta_2 = 1$, and $\alpha_2 = 1$ at upper boundary, $\beta_2 = 1$ at lower boundary	
Die-off rate in each part	
$\mu = \mu_0\theta^{T-20℃}$	(A.23)

Table A.3 Model parameters. Input parameters: parameters based on design and measurement. Calibration parameters: parameters calibrated in this study.

Water flow module parameters		**Microbial quality module parameters**	
Input parameters			
B	Length of overflow weir [L]	ρ	Bulk soil density [M L^{-3}]
P_p	Unlined perimeter [L]	λ	Dispersivity [L]
C_Q	Weir overflow coefficient [-]		
C_s	Side infiltration coefficient [-]		
K_f	Hydraulic conductivity of the surrounding material [L T^{-1}]		
S_w	Wilting point [-]: washed sand, 0.05; loamy sand, 0.07		
S_s	Saturation as the threshold for plants to reach potential evapotranspiration [-]: without SZ, 0.22; with SZ, 0.37		
S_{fc}	USZ saturation at field capacity [-]: without SZ, 0.37; with SZ, 0.61		
γ	Relative hydraulic conductivity coefficient dependent on soil type [-]: washed sand, 11.1; loamy sand, 11.76		
Calibration parameters			
K_s	Hydraulic conductivity of the filter media [L T^{-1}]	k_{att}	Adsorption rate [T^{-1}]
K_c	Plant coefficient for evapotranspiration [-]	k_{det}	Desorption rate [T^{-1}]
		μ_0	Standard die-off rate at given reference conditions (e.g., standard temperature) [T^{-1}]
		θ	Temperature correction coefficient for die-off [-]

Supplementary material B. Supplementary results of sensitivity analysis and prediction uncertainty analysis

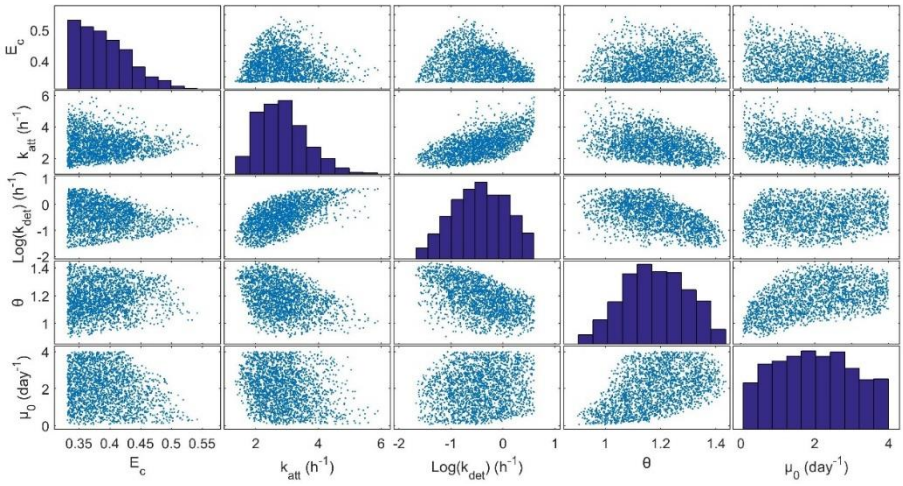


Figure B.1 Matrix plot of *Lab*-WS for sensitivity analysis.

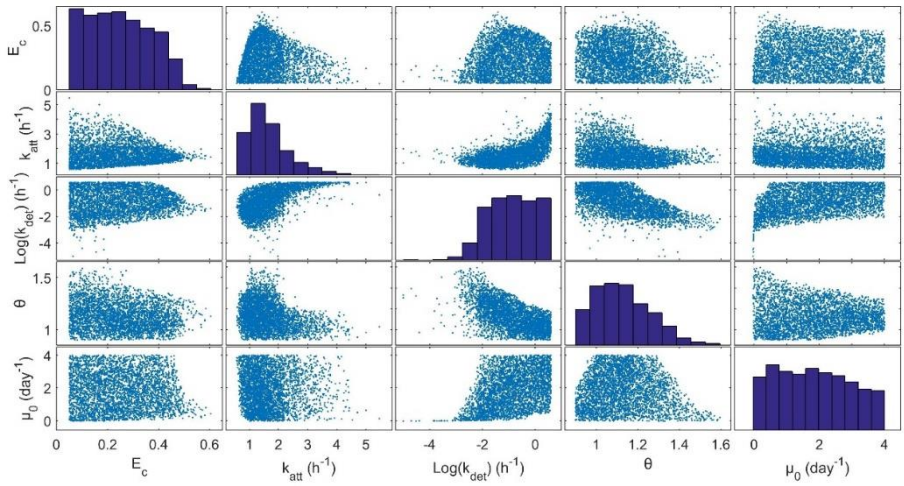


Figure B.2 Matrix plot of *Lab*-PB for sensitivity analysis.

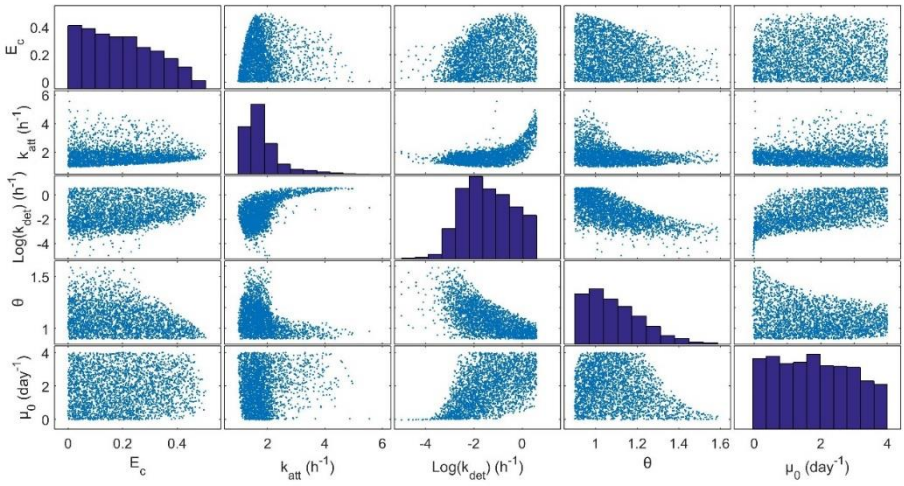


Figure B.3 Matrix plot of *Lab*-LC for sensitivity analysis.

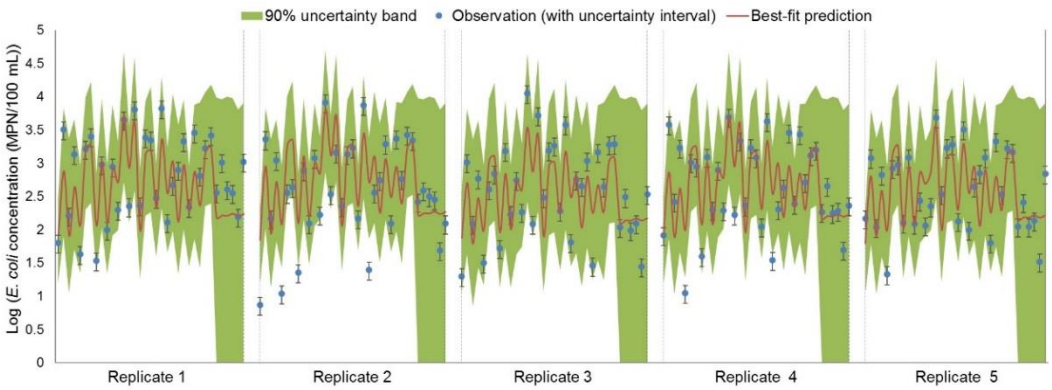


Figure B.4 Uncertainty analysis of *E. coli* removal prediction for *Lab*-WS using *Lab-12Aug*-WS parameters, including observation with uncertainty intervals, the best-fit prediction and 90% uncertainty band.

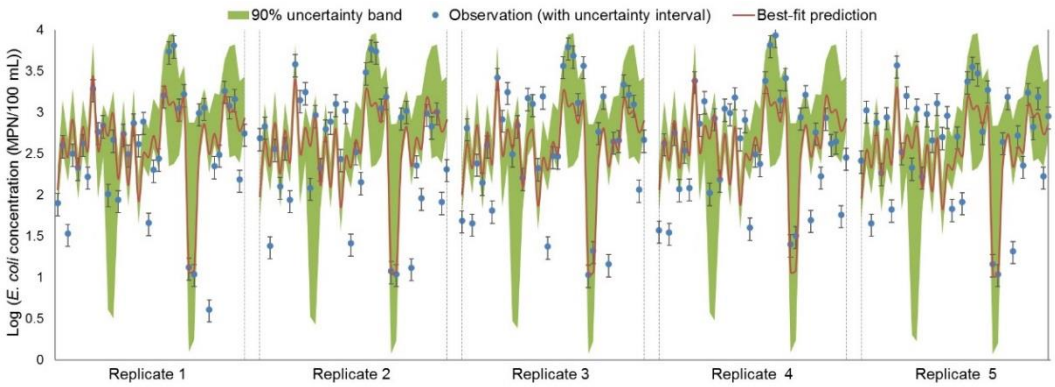


Figure B.5 Uncertainty analysis of *E. coli* removal prediction for *Lab-12Aug*-WS using *Lab*-WS parameters, including observation with uncertainty intervals, the best-fit prediction and 90% uncertainty band.

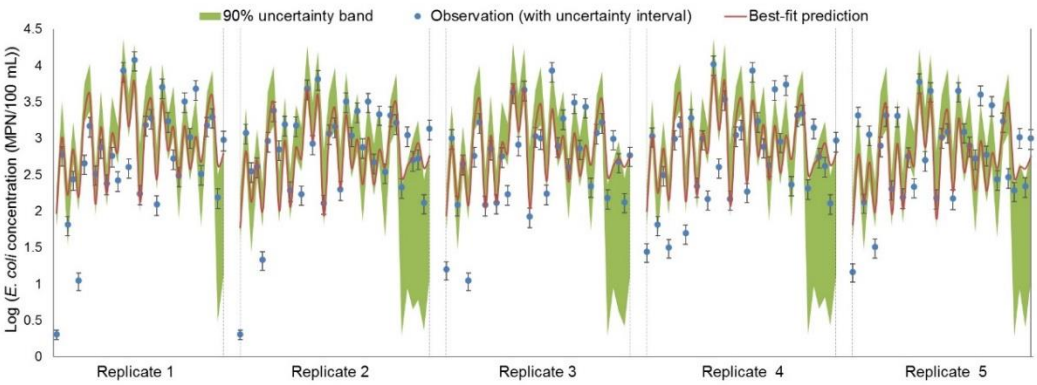


Figure B.6 Uncertainty analysis of *E. coli* removal prediction for *Lab*-PB using *Lab-12Aug*-PB parameters, including observation with uncertainty intervals, the best-fit prediction and 90% uncertainty band.

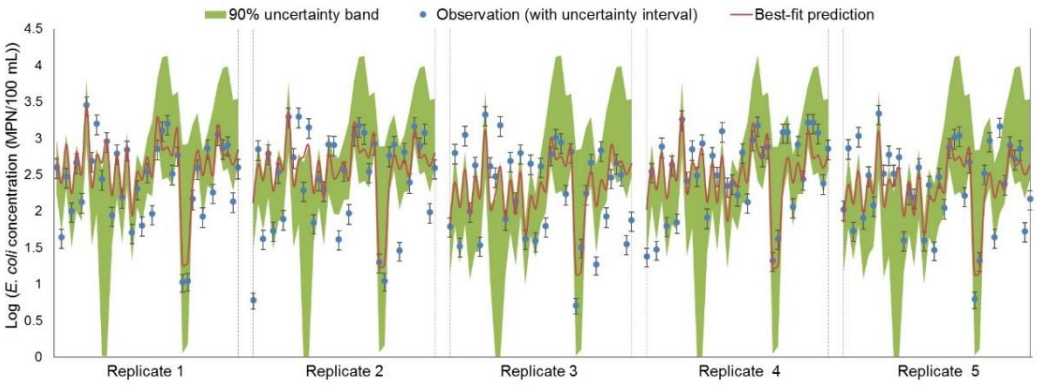


Figure B.7 Uncertainty analysis of *E. coli* removal prediction for *Lab-12Aug*-PB using *Lab*-PB parameters, including observation with uncertainty intervals, the best-fit prediction and 90% uncertainty band.

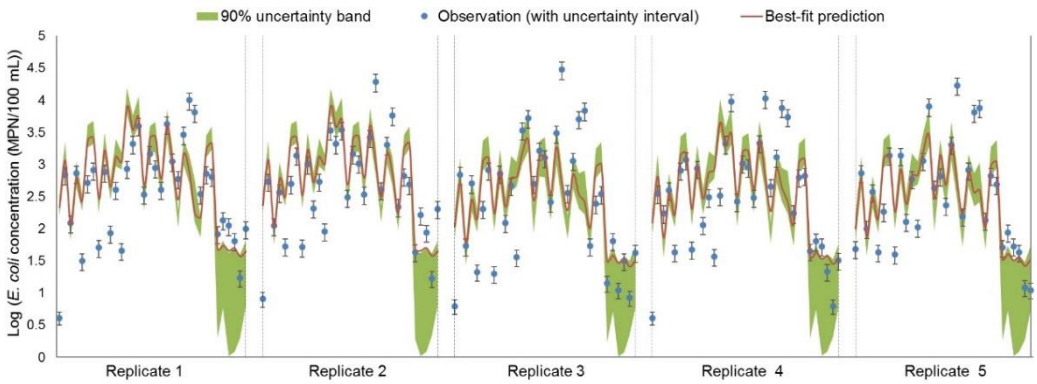


Figure B.8 Uncertainty analysis of *E. coli* removal prediction for *Lab*-LC using *Lab-12Aug*-LC parameters, including observation with uncertainty intervals, the best-fit prediction and 90% uncertainty band.

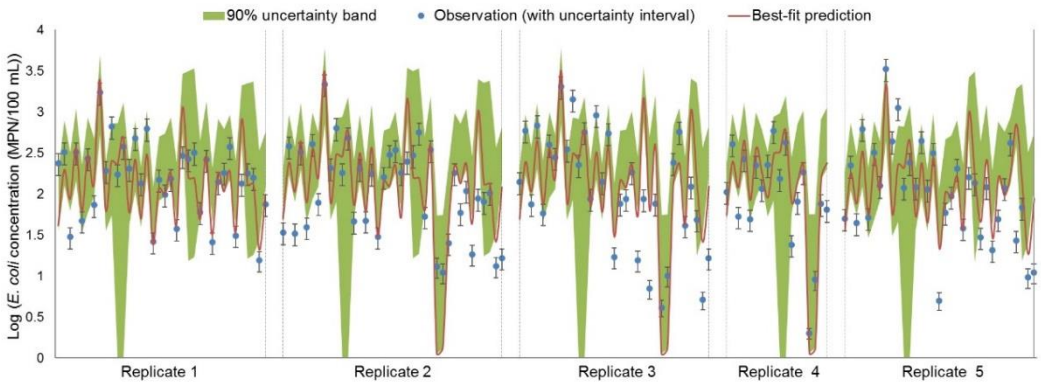


Figure B.9 Uncertainty analysis of *E. coli* removal prediction for *Lab-12Aug*-LC using *Lab*-LC parameters, including observation with uncertainty intervals, the best-fit prediction and 90% uncertainty band

4.3 Discussion and conclusions

When the previous developed model was tested with the data collected from five biofilter configurations (four lab-scale, one field-scale), the good fit between prediction and observation demonstrated the hypothesis that the governing processes (i.e., adsorption, desorption, and die-off) and key operational factor (i.e., temperature) that included in the model are adequate to provide promising microbial removal prediction. In addition, the consistent Nash-Sutcliffe Efficiency values under different biofilter design and operational conditions (especially under extreme field conditions, e.g., high inflow volume and extended dry periods) indicated that the model has a stable performance and could adjust itself to various inputs. Although the model was only developed for and applied in non-RTC biofilters (i.e., biofilters without RTC), after modification, the modified model is expected to perform well in RTC prediction, as the current model is adjustable to various operational conditions and very likely to reflect the effects of operational optimisation that was triggered by RTC.

More importantly, the high transferability of calibrated parameters and the low uncertainty in the prediction of laboratory and field systems demonstrated that the model has a broad application in both the developed systems with data collected and those with difficulty in data collection (e.g., newly developed systems; large-scale field systems). These results not only broadened the application of this model (e.g., use lab-calibrated data to test field systems), but also provided evidence that even when biofilters are modified or re-designed to incorporate RTC, the model after modification could still be applied without further calibration.

However, the model could not be used in predicting the outcome of RTC implementation, unless it is modified and validated with the data collected from RTC biofilters. Therefore, the next chapter (Chapter 5) will present a study that focuses on the experiments set up for the monitoring of RTC biofilters and RTC data collection. Chapter 6 will represent the details of modifying the developed model for RTC simulation, and employing the collected RTC data from the experiments that were introduced in Chapter 5 to test the modified model.

Chapter 5.

Development and laboratory testing of real time control strategies for stormwater biofilters

5.1 Introduction

To achieve real time control (RTC) in stormwater biofilters, RTC strategies are essential, as they directly govern how the operation of biofilters will be optimised according to biofilters' instantaneous status. However, currently there is no RTC strategy for stormwater biofilters; as such, one of the objectives in this chapter is to develop RTC strategies for stormwater biofilters.

Off-line RTC strategies (where set-points are normally fixed) are favoured for stormwater biofilters compared to on-line strategies (where set-points are variable and determined during the control process), as (1) stormwater biofilters are rather simple systems with low technology, and implementing off-line strategies requires less control facilities and easier to fulfil compared to implementing on-line strategies; and (2) an off-line strategy normally needs less time to trigger control than an on-line strategy by avoiding to re-determine set-points (Lund et al., 2018), therefore, it could provide rapid reaction to the rainfall events with short duration.

In addition, an effective RTC strategy for stormwater harvesting and reuse is expected to remove the negative impacts of extreme weather conditions, such as big events with excessive inflow, long dry periods, and short dry periods, as these three types of events could cause utmost negative effects for microbial removal (Chandrasena et al., 2012; Chandrasena et al., 2014b; Li et al., 2012). Furthermore, to ensure the feasibility and reduce the complex during RTC implementation, a RTC strategy is expected to be capable of being applied not only in newly built systems but also for the retrofit of established systems, with low facility requirements and uncomplicated control process.

Moreover, the model developed and validated in Chapter 3 and Chapter 4 has a potential to be applied in RTC strategy development and evaluation after modification, as it has been demonstrated to have high parameter transferability and low prediction uncertainty when being used to predict biofilters with various design and operational conditions. Nevertheless, the model has only been developed and tested with the data collected from non-RTC biofilters (e.g.,

typical biofilters that without RTC). Before applying the model in RTC simulation, it has to be modified and validate with RTC data. Therefore, there is a desperate need for the experimental data that collected from biofilters that incorporated with RTC to modify and test the model. As such, collecting data from laboratory testing of RTC strategies is another objective of this study.

In summary, to fulfil all these requirements to achieve RTC in stormwater biofilters, the main objectives of the study presented in this chapter are: (1) to develop effective RTC strategies for stormwater biofilters; (2) to test the developed RTC strategies with laboratory experiments, validating the outcome of RTC and providing data for modelling in further studies. The research questions include:

- What information and facilities are needed for the development and implementation of RTC strategies?
- What experiments could best reveal the effectiveness of developed RTC strategies?
- What benefits could be provided by developed RTC strategies compared to non-RTC?
- What negative effects might be caused by the implementation of RTC?

Corresponding to the research questions, four hypotheses were made:

- The information of rainfall events (e.g., rainfall forecasting, event size, and antecedent dry length) and biofilters' status (e.g., water volume in the submerged zone) are needed to trigger/stop control; facilities include valves, flowmeters, timer, and sensors are required to fulfil RTC implementation.
- The effectiveness of developed RTC strategies could be revealed by comparing the laboratory performance of RTC biofilters and non-RTC biofilters that operated under the conditions of mimicking historical rainfall events with various weather conditions (e.g., event size and antecedent dry length) and inflow concentrations.
- The developed RTC strategies will diminish the negative effects caused by extreme weather conditions, such as extensive size events, events

with extended dry period, and events with short dry period. Different RTC strategies will provide different benefits: some could provide high-quality outflow for harvesting and reuse, while some others will reduce the pollutants that discharged to ecosystems; suitable RTC strategies could be selected according to end uses and requirements.

- With RTC, the water collected for harvesting will meet the treatment objectives better compared to without RTC. However, a focus on some certain treatment objectives might cause side effects: for example, the focus of improving outflow quality of stormwater biofilters may cause additional pollutants being discharged to ecosystems; in addition, the improvement on microbial removal might diminish the treatment effects on some other pollutants such as nutrients.

This chapter presents the processes of developing two RTC strategies for stormwater biofilters, and the details of setting up laboratory experiments and experimental data collection. This chapter is written as a draft for journal publication and will be submitted to *Water Research*.

5.2 Development and laboratory testing of real time control strategies

Real time control of biofilters delivers stormwater suitable for harvesting and reuse

Pengfei Shen [a*], Ana Deletic [b] David T. McCarthy [a]

[a] Environmental and Public Health Microbiology Laboratory (EPHM Lab), Department of Civil Engineering, Monash University, Clayton, VIC 3800, Melbourne, Australia

[b] School of Civil and Environmental Engineering, University of New South Wales, Sydney, NSW 2052, Australia

[*] Corresponding author. Email address: [redacted]

Abstract

Stormwater biofilters have great potential to treat stormwater for harvesting and reuse, but their variable performance in pathogen removal requires further optimisation prior to widespread uptake. Our paper provides the first evidence that real time control (RTC) of stormwater biofilters can mitigate the impact of operational charactersitics that result in poor microbial removal. We developed two RTC strategies and validated them using long-term laboratory experiments, utilising biofilters with a raised outlet pipe that creates a submered zone. The first RTC strategy focuses on delivering the best water quality for harvesting and reuse or for recreational waterways. It has two components: (1) retains water in the biofilter for at least two days to ensure optimal die-off before allowing any further inputs into the system, and (2) when inputs are allowed to enter, the input volume is restricted to the submerged zone's pore volume. This strategy was effective and signficantly improved water quality in the biofilter effluent. However, since the system favours bypassing influent to ensure good quality effluent, only 28.4 % of the stormwater was treated, which still resulted in a 62.3 % reduction in the influent *E. coli* load because the system was

effective at removing *E. coli* under controlled conditions. The second RTC strategy builds upon the first strategy, but focuses on delivering a balance between good water quality for harvesting and protecting the environment (i.e. lower bypass). This strategy also has two components: (1) three hours before next rainfall event begins, the water that has remained in the biofilter's submerged zone for at least two days is drained and collected for harvesting through a bottom pipe, and (2) when stormwater inflow begins, the bottom pipe is closed and the biofilter operates without control as a normal biofilter, with water leaving the biofilter to the environment via the raised outlet pipe. The bottom pipe's effluent of this RTC strategy met the Australian stormwater harvesting guideline requirements for dual reticulation with indoor and outdoor use and irrigation of commercial food crops. Although only 5.4 % of stormwater was collected for harvesting via the bottom pipe in this strategy, the environment was better protected because of a signficnatly reduced bypass volume. Our exepriments also showed nutrient and sediment removal was high for both RTC strategies. This study presents the first stepping stone toward RTC of stormwater biofilters, demonstrating that these systems can deliver safe water for harvesting and reuse, and for active recreational uses.

Keywords: stormwater biofilters, real time control, stormwater harvesting, stormwater reuse, microbial removal, *E. coli*

1. Introduction

Stormwater runoff could serve as an alternative water resource for local communities (Mitchell et al., 2007); however, the wide range of pollutants contained in stormwater runoff impede stormwater harvesting and reuse (Fletcher et al., 2008). In particular, faecal microbes contained in stormwater are a major concern, as they could pose considerable risks to human health (Meng et al., 2018; Murphy et al., 2017). As such, several guidelines of stormwater harvesting and reuse have been established in Australia and globally to strictly control the risks posed by faecal microbes under different end uses (NHMRC, 2008; NHMRC, 2009).

On way to control the risks is to treat stormwater, and stormwater biofilters have been widely adopted to achieve this aim (Bratieres et al., 2008; Hathaway et al., 2011; Hatt et al., 2009; Zinger et al., 2011). Stormwater biofilters are soil-plant systems with enhanced infiltration and evapotranspiration (FAWB, 2009). The major processes for microbial removal in biofilters include adsorption, desorption, straining and die-off, and these processes are also governed by various factors (e.g., temperature, moisture content, and sunlight exposure) (Chandrasena et al., 2014a; Stevik et al., 2004).

Biofilters have better faecal microbial removal than other water sensitive urban design systems (e.g., wetlands) (Chandrasena et al., 2012; Hathaway and Hunt, 2010), somewhat owing to the level of research that has been dedicated to optimise their design features. Indeed, much effort has been placed in optimising biofilters' design: for example, microbial removal can be enhanced through careful plant and media selection (Chandrasena et al., 2014b; Li et al., 2016; Li et al., 2012) and the presence of a submerged anoxic zone (i.e., a submerged water body at the base of a biofilter to help plants survive in dry weather periods and to enhance microbes' contact time with media) (Chandrasena et al., 2014b).

However, even with optimal designs, stormwater biofilters still experience variable performance. For example, *E. coli* reductions from the inlet to outlet could vary between > 99 % to net leaching (< 0 %) under challenging operational conditions of a well-designed biofilter (Chandrasena et al., 2014b; Li et al., 2012; Zhang et al., 2010; Zhang et al., 2011). Further examples existed for other reference pathogens such as *Campylobacter* spp.: e.g., Chandrasena et al. (2016) reported its removal rate ranged from -90.5 % to 99.1 %. The major explanation for these high variances is, the optimisation of design could not mitigate the negative impact of some operational conditions.

According to previous studies, three types of operational conditions mainly contributed to poor pathogen removal via stormwater biofilters. (1) Short dry weather periods between wet weather events (e.g., < 12 h), which could result in insufficient time for die-off of microbes trapped in the system, thereby causing

detachment and leaching of viable organisms to the outlet, even if the influent is clean (Chandrasena et al., 2012). This is especially apparent for microbes that are trapped in the submerged zone water, which if left for sufficient period can produce extremely clean water at the effluent when being pushed out by the newly entered water. (2) Large inflow volumes, which could result in full occupation of straining and adsorption sites in biofilters, thereby leading to breakthrough of microbes. This could be further exacerbated by diluting the clean submerged zone water with newly applied influent, resulting in poorer removal rates (Chandrasena et al., 2014b). (3) Long dry weather periods between events (e.g., ≥ 14 days), which could result in poorer plant health (a key design parameter for pathogen removal, as per Li et al. (2012)) and the emptying of the clean submerged zone water via plant uptake, leading to the effluent of the subsequent event containing a high proportion of new influent that has had limited retention time (Chandrasena et al., 2014b).

Although the operational conditions described above are recognised as governing much of the microbial removal performance, very little has been done to control them to eliminate their negative effects, as stormwater biofilters have primarily been designed as passive systems. To make biofilters “active” for the optimisation of their operation conditions, real time control (RTC) is a potential technique. The general concept of RTC is defined as: monitoring the functioning of a system in real time, and using these data to optimise performance through the control of certain aspects of the system (Schütze et al., 2004). RTC has been widely used and proved to be effective in other studies in environmental field, such as flooding control, capacity enlargement of sewer systems, and wastewater treatment optimisation (Hsu et al., 2015; Leon et al., 2014; Schilling et al., 1996; Schütze et al., 2004). However, no publication has been found on the application of RTC in stormwater biofilters.

The main objective of this study is to develop and validate the first reported RTC strategies for the optimisation of stormwater biofilters, in order to demonstrate that the regulation and control of certain operational conditions can lead to enhanced faecal microbe removal. In this study, we aim to provide better water quality using biofilters and to reduce the human health risks posed

by faecal microbes during stormwater harvesting and reuse. To achieve this aim, two different RTC strategies were developed and tested using laboratory experiments, mimicking real rainfall events and drying patterns that were recorded in Melbourne, Australia.

2. Methods

2.1 RTC Strategy development

The RTC strategies should avoid the three operational characteristics explained in the introduction that lead to poor pathogen removal: (a) short dry weather periods, (b) long dry weather periods, and (c) large inflow volume events. Using this knowledge, the following objectives were devised:

Objective 1 - the strategies should attempt to promote the detention of water in the system for sufficient time (≥ T_{min}; T_{min} is the minimum retention time that required for sufficient die-off) to allow optimal microbial die-off, but not excessively long that the water contained in the submerged zone is removed by plants (as per Chandrasena et al. (2014b));

Objective 2 - these strategies should collect the water that retained in stormwater for sufficient time, as these water is of high quality; in addition, this these water should avoid being mixed with newly treated water(which is normally of low quality due to short contact time), by ensuring that no excessive water from large events enter the system;

Objective 3 - the strategies should be flexible in its implementation, allowing for easy system design and facility installation, both for new systems and for retrofit scenarios (meaning only surface accessible equipment can be installed);

Objective 4 - the strategies that require low maintenance and low cost equipment (e.g., sensors) are favoured; hence, low-technical solutions are preferable; and,

Objective 5 - The strategies are expected to deliver on both stormwater harvesting outcomes and environmental protection (e.g., minimum pathogens for swimming, and low nutrients for ecosystem health).

Based on these objectives, two RTC strategies were developed in this study: (a) Harvesting RTC, and (b) Harvesting-Environment RTC. Both strategies are described in detail below in Section 2.1.1 and Section 2.1.2.

2.1.1 Harvesting RTC

The Harvesting RTC strategy shown in Figure 1 collects as much clean water as possible for the purpose of harvesting and reuse or for delivery into recreational waterways. To deliver on Objective 1 (listed in Section 2.1), this strategy controls the inflow by passing it around the system, unless the water from previous event has remained in biofilters for at least T_{min} (Figure 1 top right and bottom left). To deliver on Objective 2, this strategy also ensures that no excessive water from large events enter the system, by bypassing any volume during an event that has exceeded the pore volume of submerged zone (V_{sz}) (Figure 1 bottom right).

To deliver on Objective 3 and Objective 4, this RTC strategy allows for easy retrofits, with no below-surface amendments nor any within-system monitoring. The only facilities required for this RTC strategy are (Figure 1 - top left): (1) a flow meter (or depth gauge with weir) to estimate inflow volumes (V_{in} in Figure 1), (2) a valve to control stormwater entering/bypassing the system, and (3) a timer to monitor the time that the water from previous event has retained in the system (T in Figure 1).

It is noted that this strategy only delivers on the stormwater harvesting outcomes in Objective 5, by aiming to maximise the volume of collected water that has had sufficient retention time. This strategy places minimal emphasis on environmental protection, as part of the incoming water is bypassed without treatment (Figure 1).

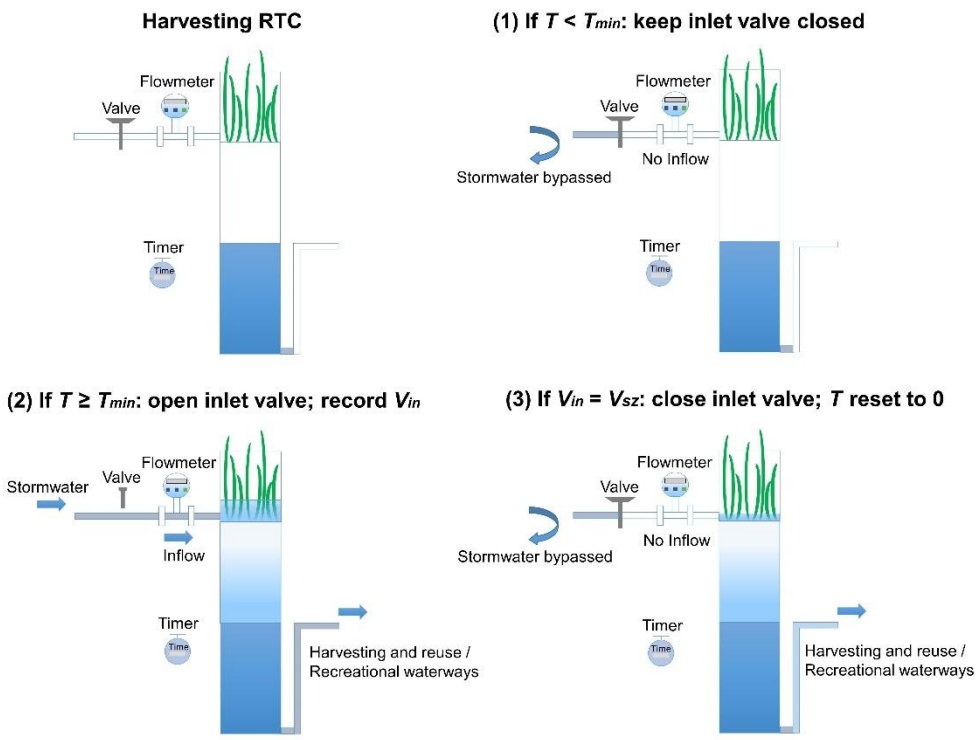


Figure 1 Schematic of Harvesting RTC's rules. T: stormwater retention time; T_{min}: minimum required retention time; V_{in}: stormwater volume that has entered biofilters; V_{sz}: submerged zone's pore volume (V_{sz} = biofilter's bottom area × submerged zone depth × porosity). Top left shows the required facilities to fulfil this strategy (i.e., a flow meter, a valve, and a timer). When stormwater runoff comes: if $T < T_{min}$, keep the valve closed and bypass all the stormwater (top right); if $T \geq T_{min}$, open the valve and start to record V_{in} (bottom left). When $V_{in} = V_{sz}$ (i.e., submerged zone's pores will be fully replaced with newly entered stormwater), close the valve and bypass the rest of stormwater (bottom right).

2.1.2 Harvesting-Environment RTC

The Harvesting-Environment RTC attempts to build upon the first strategy, and enhances it to overcome the fact that the Harvesting RTC strategy favours bypassing large volumes of untreated stormwater into receiving water bodies. A more complicated strategy is developed by proposing a two-outlet system (Figure 2 top left): the valve for the bottom outlet is opened only if the water has been held for more than T_{min} and inflow is forecasted to occur in the near future (e.g., 3 hours) (Figure 2 top right and bottom left). This water from the bottom outlet meets Objective 1 and Objective 2, delivering clean harvestable water to a dedicated store or recreational waterway. Once stormwater inflow begins, the

bottom valve is closed, the influent will fill the system and the biofilter begins to operate as typical (i.e., without control), with treated effluent leaving via the raised outlet and being delivered to receiving waterbodies (to meet Objective 5; Figure 2 bottom right). Although this strategy is more advanced, it still meets Objective 3 and Objective 4 in several ways. The system does not require flow monitoring equipment for inflow and only a sensor (e.g., water level sensor) is required at the inflow pipe to provide a binary YES/NO regarding the occurrence of inflows (Figure 2 top left). The system is completed with a simple valve at the bottom of the column (a manual valve is often included in a typical biofilter for maintenance reasons), and a timer to ensure T_{min} is met (Figure 2 top left). The only other piece of information required for this strategy is rainfall forecast. In Melbourne and many other cities around the globe, 3-hourly forecasts are available for free from local weather bureaus (e.g., http://www.bom.gov.au/australia/meteye/); this information can communicate to biofilters via telemetry. In addition, three hours are generally long enough to discharge the water retained in biofilters through the bottom pipe (FAWB, 2009). As such, in this study, the rainfall forecast information is assumed to be 3-hourly. However, this could also be changed in another study, according to the available information for rainfall forecast and biofilters' inherent features.

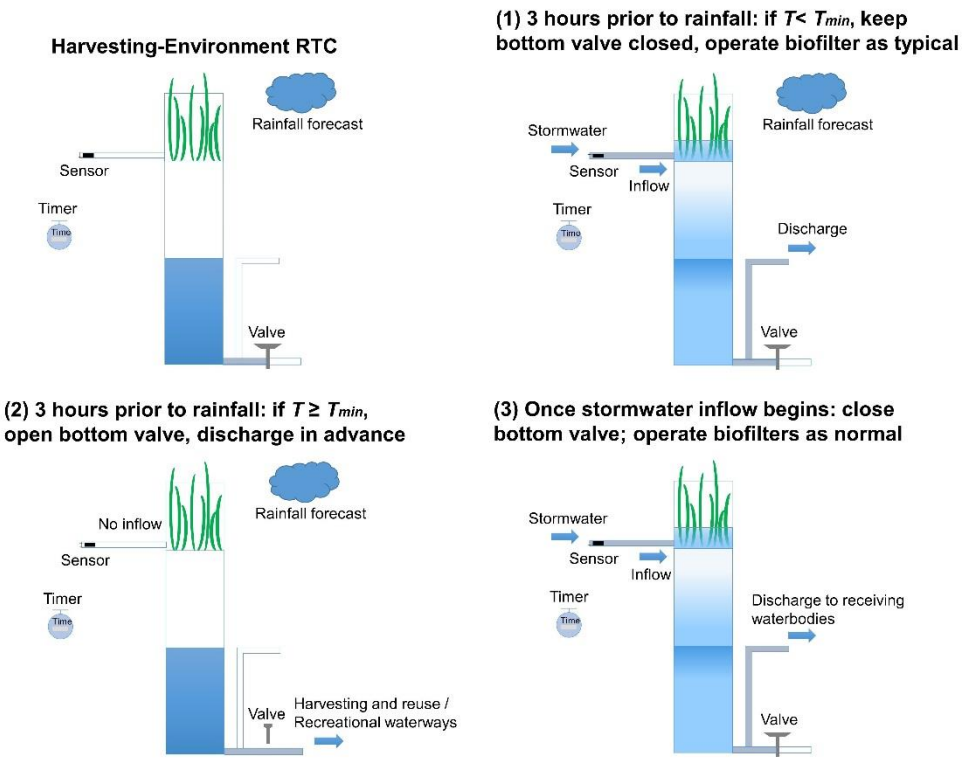


Figure 2 Schematic of Harvesting-Environment RTC's rules. T: stormwater retention time; T_{min}: minimum required retention time. Top left shows the required facilities and information to fulfil this strategy (i.e., a sensor, a valve, a timer, and rainfall forecast). When rainfall forecast informs that the next wet event will occur in three hours: if $T < T_{min}$, keep the valve closed and operate biofilters as typical, and the treated stormwater is discharged through the raised outflow pipe (top right); if $T \geq T_{min}$, open the valve and collected the harvestable water through free draining (bottom left). Once stormwater inflow begins, biofilters operate as typical again (bottom right).

2.2 Laboratory set-up

2.2.1 Experimental biofilter columns

These two RTC strategies were assessed using a laboratory column study. Ten biofilter columns were planted with *Carex appressa*, using washed sand as the filter media. These columns had an identical size (Figure 3). Although five of them had wood chips in the submerged zone (5 % by volume) to add carbon source and the other five did not, it has been reported that the existence of wood chips had no significant impact on microbial removal (Jung et al., 2019). These ten columns were randomly divided into two groups with five replicates

each: RTC biofilters (on which RTC strategies were implemented) and non-RTC biofilters (operated as typical biofilters without RTC). To avoid any potential bias, the columns with and without woodchips were divided into each group: two woodchip and three non-woodchip columns for RTC biofilters, while three woodchip and two non-woodchip columns for non-RTC biofilters. We observed no obvious difference in the performance between the two types of columns, and hence these two types are assumed to be replicates of one another for the purposes of this research.

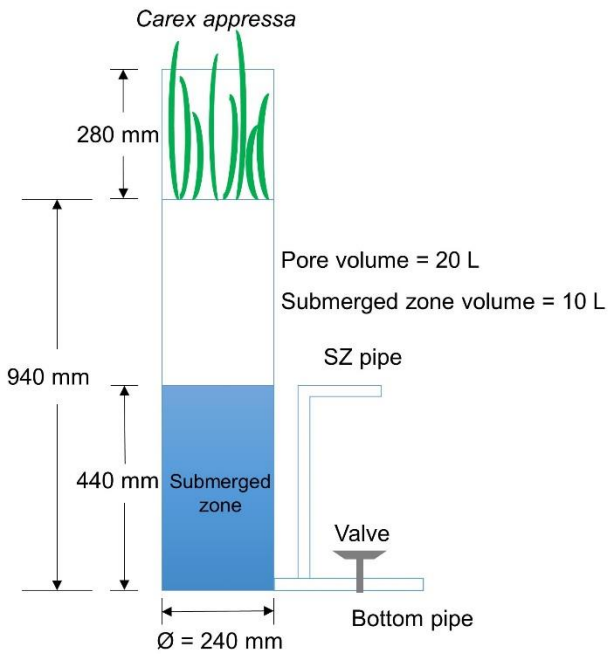


Figure 3 Schematic of a biofilter column in laboratory experiments.

It is important to note that these columns were matured for approximately three years prior to use in this project and each column had been previously fed with 866 L of semi-natural stormwater and 2910 L of synthetic greywater and results were previously presented by Fowdar et al. (2017) and Jung et al. (2019). It is important to note that (1) all columns prior to the start of the experiments in this study had received the same amount and type of input, and (2) the dataset within this manuscript has not been presented elsewhere. Furthermore, prior to use in this project, all the biofilters were further dosed with a semi-natural stormwater mix (see Section 2.2.2), twice per week (corresponding to the rainfall frequency in Melbourne) for three months, allowing plants to grow and acclimatise to this experiment's operational conditions. Each time, the dosed

volume for each column was 13 L, representing the average rainfall event size (5.75 mm) in Melbourne and assuming the biofilters are sized to 2% of their impervious catchment area (FAWB, 2009).

2.2.2 Semi-natural stormwater mix

The semi-natural stormwater used for dosing was prepared by mixing de-chlorinated tap water with sediments collected from a local stormwater wetland, supplementing with laboratory grade chemicals, and spiking with *Escherichia coli* (*E. coli*, as a faecal microbial indicator), to target the 'average' pollutant concentrations reported in a worldwide review of stormwater quality (Duncan, 1999; McCarthy et al., 2008; NHMRC, 2009). The processes and recipe for semi-natural stormwater preparation were the same as those employed by Chandrasena et al. (2017).

2.2.3 Design of semi-natural stormwater events

Dosing volumes and dosing concentrations of *E. coli* varied during events when the inflow and outflow of each column were sampled to determine pollutant removal efficiency. Eleven Sampling events were designed to simulate 11 selected historical rainfall events in Melbourne (from 2007 to 2016). To fully assess RTC under different weather conditions, these selected events were of three typical event sizes (Table): (1) large size (V_L) with around 17.68 mm (equivalent to 40 L dosing volume, which is 2 pore volumes of a column) per event, which represents a 1 in 3 month average recurrence interval (ARI); (2) median size (V_M) with around 8.84 mm (20 L dosing volume; 1 pore volume) per event, representing 1 in 1 month ARI; (3) small size (V_S) with around 4.42 mm (10 L dosing volume; same as the submerged zone volume) per event. These 11 events also have three types of antecedent dry lengths (Table): (1) long dry (D_L), with ≥ 14 antecedent dry days; (2) short dry (D_S), with < 2 antecedent dry days; (3) median dry (D_M), in which 2 days ≤ antecedent dry days < 14 days. Similarly, during laboratory experiments, three types of inflow concentrations were designed when spiking *E.coli* in semi-natural stormwater (Table): (1) high/large concentration (C_L), with a magnitude of 10^5 MPN/100 mL; (2) low/small concentration (C_S), with a magnitude of 10^4 MPN/100 mL; (3) median concentration (C_M), with a magnitude of 10^2 MPN/100 mL.

Table 1 Characteristics of each sampling event, including event size, inflow concentration, and antecedent dry length.

Event No.	1	2	3	4	5	6	7	8	9	10	11
Dosing volume (L)	39.8	39.4	40.3	37.6	9	10	9	18.5	20.4	21.7	22.2
Size type	V_L	V_L	V_L	V_L	V_S	V_S	V_S	V_M	V_M	V_M	V_M
Antecedent dry days	3	1	2	14	4	8	1	2	5	1	21
Dry type	D_M	D_S	D_M	D_L	D_M	D_M	D_S	D_M	D_M	D_S	D_L
Inflow concentration (MPN/100 mL) in Round 1	3.11×10^4	1.34×10^2	3.22×10^4	3.76×10^4	3.11×10^5	2.28×10^5	1.58×10^2	1.44×10^5	2.25×10^4	1.35×10^4	1.12×10^4
Inflow concentration (MPN/100 mL) in Round 2	2.14×10^4	1.61×10^2	1.04×10^4	1.66×10^4	5.48×10^5	6.89×10^5	6.89×10^2	7.06×10^5	6.87×10^4	6.83×10^4	6.49×10^4
Concentration type	C_M	C_S	C_M	C_M	C_L	C_L	C_S	C_L	C_M	C_M	C_M

2.2.4 Implementation of RTC strategies

To assess the two RTC strategies, two rounds of experiments were conducted. In Round 1, Harvesting RTC was implemented for all 11 sampling events. In Round 2, these sampling events were repeated with the implementation of Harvesting-Environment RTC (Table 1). In this study, for both RTC strategies, the minimum required retention time for the water contained in biofilters (T_{min}) was set as two days, according to the findings reported in Chandrasena (2014). However, a different set point could be applied to balance competing objectives.

For Round 1 (Harvesting RTC), in each sampling event, dosing was stopped for RTC biofilters when the inflow volume (V_{in}) reached the submerged zone volume (V_{sz}; = 10 L); for the events had short antecedent dry periods (< T_{min}; i.e., < 2 days), no water was dosed into RTC biofilters.

In Round 2, it is assumed that perfect rainfall forecasting information was available (i.e., no uncertainties were caused by inaccurate forecast). This assumption allowed simplicity in implementation, and could fully reveal the benefits of Harvesting-Environment RTC. In addition, no historical rainfall forecast archive was available for this study, and future work will be conducted to explore the uncertainties caused by inaccurate rainfall forecast. For this round, sampling events that had an antecedent dry weather period of T_{min} or greater, the water retained in RTC biofilters was discharged in advance through

the bottom pipe, and this discharge started three hours prior to dosing. In sampling events with short dry periods (< T_{min}), the bottom pipe was closed, and RTC biofilters were operated as typical. Regardless of event type, when dosing started, these biofilters were operated the same as non-RTC biofilters.

During both Round 1 and Round 2, non-RTC biofilters were also used, and operated as traditional biofilters without any control, receiving and treating all the dosed stormwater. These served as experiment controls for comparison to the RTC biofilters.

2.2.5 Sampling regime

During each sampling event, inflow was sampled at the beginning, middle, and end of the sampling period; these were mixed to create a composite inflow sample. In each sampling event of Round 1 (Harvesting RTC), a sub-sample was collected from all of the composite outflow that drained from the RTC biofilters, and this sample was named “RTC-harvesting”. The water that bypassed to the environment was assumed to have the same concentration as the inflow, and was named as “RTC-environment (bypassed)” (Figure 4). For non-RTC biofilters, a sub-sample was collected from the outflow and named as “non-RTC outflow” (Figure 4).

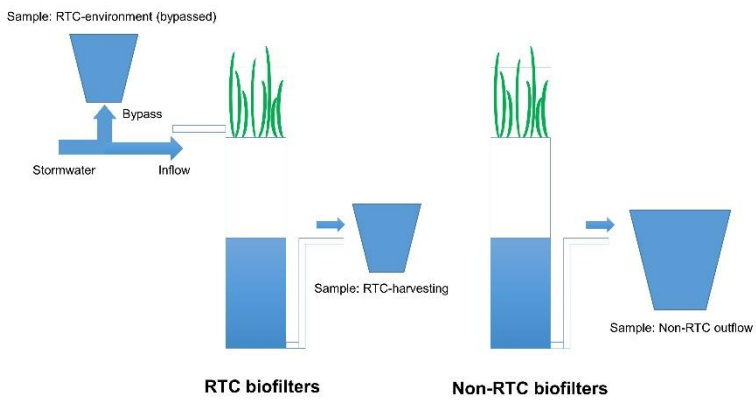


Figure 4 Collection of outflow samples in Round 1 (Harvesting RTC).

For RTC biofilters in Round 2 (Harvesting-Environment RTC), a sub-sample was collected from the composite water that was discharged in advance (for harvesting), naming as “RTC-harvesting”; another sub-sample was extracted from the composite outflow when RTC-biofilters was operated as typical

(discharged into the ecosystem after treatment), naming as “RTC-environment (treated)” (Figure 5). For non-RTC biofilters, a sub-sample was collected from the composite outflow and named as “non-RTC outflow” (Figure 5).

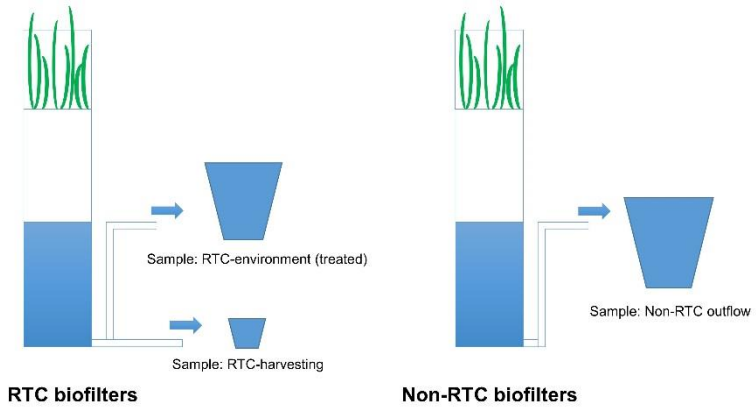


Figure 5 Collection of outflow samples in Round 2 (Harvesting-Environment RTC).

All samples were transported on ice to the Environmental and Public Health Microbiology Laboratory (EPHM Lab) for enumeration of *E. coli* using the Colilert method™ (IDEXX-Laboratories, 2007) within 8 hours of dosing. According to McCarthy et al. (2008), this storage time would cause no significant difference in *E. coli* concentrations. Turbidity was also tested for each sample by using Orion™ AQUAfast™ AQ4500 Turbidity Meter.

In each round of experiments, samples were collected from the last three events (Event 9, Event 10 and Event 11) and tested for TSS (total suspended solids), TP (total phosphorus) and TN (total nitrogen) in NATA (National Association of Testing Authorities, Australia) accredited laboratories using standard methods (TSS: Method 2540 D in APHA-AWWA-WEF (2005); TP and TN: Method 4500-P J in APHA-AWWA-WEF (2005)), to analyse the impact of RTC on the removal of these pollutants.

The outflow volumes of each event for each column was measured. The evapotranspiration volume for each round was estimated through mass balance: e.g., evapotranspiration volume = inflow volume – outflow volume – bypassed volume. During each sampling event, the instantaneous water depths

in the ponding zone were recorded after regular intervals (3.75 minutes) to estimate infiltration rates.

2.3 Data analysis

Removal of *E. coli* was presented in the form of log reduction, which was the difference between the logarithmic (base 10) inflow concentration and the logarithmic outflow concentration. The data of *E. coli* log reduction and infiltration rate were analysed for normality using the Shapiro-Wilk test; the results showed that the infiltration rate data and the *E. coli* log reduction data for RTC biofilters were all normally distributed, while the *E. coli* log reduction data for non-RTC biofilters were non-normally distributed. Although non-parametric analysis is generally recommended for non-normally distributed data, the available non-parametric analytical methods are limited in testing the influence of single factor rather than the possible interactions between different individual factors (Kirk, 2008). In addition, even when the normality assumption is not verified, analysis of variance (ANOVA) is still considered as a robust test if a low p-value (e.g., $p < 0.001$) could be achieved (Kirk, 2008). As such, a series of ANOVA along with post hoc tests (Tukey HSD) was performed for pollutants removal, infiltration rate, and temperature comparisons. A three-way ANOVA was conducted to study whether the observed *E. coli* log reductions were affected by dry period length, event size, or inflow concentration. Two more three-way ANOVA were carried out to analyse whether the *E. coli* concentrations / the log reductions of *E. coli* concentration were affected by the biofilter type (i.e., RTC or non-RTC), Round, and Event.

In each round, for each type of biofilters, several calculation were made as: (1) proportion of harvested stormwater volume = volume of harvested water / total stormwater volume (all the stormwater inflow, including the bypassed water); (2) proportion of bypassed stormwater volume = volume of bypassed water / total stormwater volume; (3) proportion of discharged stormwater volume = volume of water discharged to the environment after treatment / total stormwater volume; (4) proportion of stormwater evapotranspired = 1 - proportion of harvested stormwater volume - proportion of bypassed stormwater volume - proportion of discharged stormwater volume; (5)

proportion of bypassed stormwater load = load in the bypassed water / total load in the stormwater (all the stormwater inflow); and (6) total load removal rate = 1 – (load in the harvested stormwater - total load in the bypassed stormwater - load in the discharged stormwater) / total load in the stormwater. It is noted at, we calculated the total load removal rate in a conservative way, because if the water for harvesting is directly conveyed for reuse, the load in it will be deferred from the stormwater system, and the part "load in the harvested stormwater" could therefore be removed from the calculation.

3. Results

3.1 Overall performance of RTC

3.1.1 Round 1: Harvesting RTC

The median *E. coli* concentration of RTC-harvesting (i.e., the water collected from RTC biofilters for harvesting) was 9.3 times lower than that of the non-RTC outflow. In addition, RTC-harvesting met the requirement for secondary contact recreation in Australia (median concentration < 1000 *E. coli*/100 mL; NHMRC (2008)), while non-RTC outflow did not. Furthermore, the median log reduction of RTC-harvesting was 2.7 times higher than non-RTC outflow (
Table). The 5th percentile log reduction of RTC-harvesting was 1.01, meaning that in the vast majority cases, at least 1 log reduction could be achieved for RTC biofilters. The negative value of the 5th percentile log reduction for non-RTC outflow (-1.75) was likely from the short dry events, in which the trapped microbes were flushed out by the inflow with low microbial concentrations before experiencing sufficient die-off (similar to what Chandrasena et al. (2012) found).

Importantly, for RTC biofilters, only 40 % volume of the stormwater was filtered, and the rest was directly bypassed without treatment to the environment (median *E. coli* of 2.68 × 10^3 MPN/100mL), while 100 % of the stormwater was treated by non-RTC biofilters (median *E. coli* of 6.87 × 10^3 MPN/100mL). The *E. coli* load reductions in the RTC biofilters were 62 % as compared to 83 % for the non-RTC biofilters. These values did not follow the proportion bypassed/treated because of the more efficient treatment of the RTC columns for the harvested portion of water.

Table 2 Overall performance of RTC and non-RTC biofilters with the implementation of Harvesting RTC (Round 1) and Harvesting-Environment RTC (Round 2).

		Round 1: Harvesting RTC			**Round 2: Harvesting-Environment RTC**		
Median inflow concentration (5th percentile, 95th percentile) (MPN/100 mL)		3.09×10^4 (145, 2.69×10^5)			6.46×10^4 (331, 6.92×10^5)		
		RTC biofilters		**Non-RTC biofilters**	**RTC biofilters**		**Non-RTC biofilters**
		RTC-harvesting	RTC-environment (bypassed)	Non-RTC outflow	RTC-harvesting	RTC-environment (treated)	Non-RTC outflow
Median outflow concentration (MPN/100 mL)		740	2.68×10^4	6.87×10^3	< 1	6.50×10^3	5.83×10^3
Log reduction	Median (5th percentile, 95th percentile)	1.81 (1.01, 2.71)	0 (0, 0)	0.66 (-1.75, 2.66)	4.94 (2.92, 5.85)	0.88 (-1.06, 1.67)	0.90 (-1.01, 1.76)
% stormwater volume harvested		28.4 %		0 %	5.40 %		0 %
% stormwater volume bypassed		60.0 %		0 %	0 %		0 %
% stormwater evapotranspired		11.6 %		10.8 %	11.4 %		11.9 %
% total load removal		62.3 %		83.1 %	93.7 %		92.8 %
% stormwater load bypassed		36.6 %		0 %	0 %		0 %

The impact of antecendent dry length, event size, and inflow concentration on harvesting was also analysed, based on the detailed experimental results for each event that listed in the supplementary material (Figure A.2 and Figure A.3). Event size did not impact the volume or quality of RTC-harvesting (p = 0.481, three-way ANOVA), as in each event, the volume of inflow that allowed to enter RTC biofilters was exactly the submerged zone volume. However, both antecedent dry length inflow concentration impacted the water quality for harvesting (p = 0.003 and 0.023, respectively; three-way ANOVA). The quality of RTC-harvesting samples collected after long dry periods was lower than

those collected after median dry periods, as the low moisture content in biofilters after long dry periods increased potential preferential flow pathos (as confirmed by increased infiltration rates; Figure A.1 in the supplementary material), and accordingly the likelihood of the retained stormwater being better mixed with newly applied stormwater. After short dry periods, no water was collected for RTC-harvesting as the retention time of retained water was insufficient and all stormwater was therefore bypassed. Similarly, RTC-harvesting was of low quality during high concentration inflow events compared to that collected in median concentration inflow events, mainly due to the water retained from previous event was mixed with newly entered stormwater, and higher concentration inflow caused lower quality after mixture. However, only 5.4 % of the total stormwater volume was harvested for RTC biofilters (
Table), mainly because of the soil-holding capacity of biofilters not allowing all the pore water to drain freely.

Since this RTC strategy continues to treat the incoming stormwater after the harvesting has finished, the water quality released to the environment from RTC biofilters (RTC-environment) was similar to that of the non-RTC outflow (p =0.926; one-way ANOVA), indicating that the implementation of Harvesting-Ecosystem RTC would not exacerbate the pollution on ecosystems. Furthermore, 100 % of the stormwater was treated by both RTC and non RTC biofilters in Round 2, meaning that the load reductions were comparable (93.7 % vs. 92.8 %) (Table 2).

Neither event size nor inflow concentration were significant factors for RTC-harvesting (p = 0.761 and 0.179, respectively; three-way ANOVA). Since RTC-harvesting entirely came from the previous event's water, it only depended on (1) the water volume and quality in previous events, and (2) the length of the antecedent dry weather period. Indeed, for this strategy, dry weather length impacted the volume and quality for harvesting. This was because all the water for harvesting was collected from the events with median dry periods. No RTC-harvesting samples were collected after long dry periods, as biofilters had very low moisture content after the extensive evapotranspiration, and the water contained in the pores were all held by the filter media. Also, during short dry

events, in order to maintain minimum retention times for harvested water, RTC biofilters were only operated as typical and hence no RTC-harvesting samples were collected. It is noted that, although no water was collected for harvesting, it was still better than not collecting any water with low quality, so that the water quality for harvesting could be consistently kept high.

3.2 Comparison of two RTC strategies

Although in the three-way ANOVA of the log reductions of *E. coli* concentration, Round is a significant factor ($p < 0.001$), significant interaction was found between Round and biofilter type ($p < 0.001$), and Round and Event ($p < 0.001$). Similar results were found in the three-way ANOVA of *E. coli* concentrations. Actually, when comparing the overall performance of non-RTC biofilters in Round 1 with that in Round 2 after the elimination of the interactive factors, there was no significant difference, as comparable log reduction ($p = 0.353$; one-way ANOVA) and *E. coli* concentration ($p = 0.394$; one-way ANOVA) in non-RTC outflow were observed in the rounds. In addition, there were no significant difference between the inflow concentrations of *E. coli* in the two Rounds ($p = 0.187$; one-way ANOVA), although they were not exactly the same due to the continuous *E. coli* growth/die-off during spiking. Therefore, it is proposed that the fact that we implemented the two strategies in series instead of in parallel caused no significant impact to the performance of RTC biofilters in Round 1 and in Round 2, enabling a comparison between the two strategies.

Compared to Harvesting RTC, Harvesting-Environment RTC delivered both higher quality of harvested water (median *E. coli* concentration: < 1 MPN/100 mL vs. 740 MPN/100 mL) and higher quality of environmental discharges (total load removal: 93.7 % vs. 62.3 %) (Table 2). The higher water quality of harvested water collected from Harvesting-Environment RTC biofilters than in Harvesting RTC biofilters was mainly because, when collecting the water for harvesting, in Round 1 stormwater was entering the Harvesting RTC biofilters in the meantime and creating a ponding on the top of biofilters, while in Round 2 no water was entering biofilters and therefore Harvesting-Environment RTC biofilters have a much lower hydraulic head compared to those in Round 1. As such, the outflow rates of harvested water in Round 1 were higher than those

in Round 2, resulting in a higher desorption of the microbes that were trapped in the biofilter media and plant roots. In addition, the harvested water collected from Harvesting-Environment RTC through free draining only contained clean water with sufficient die-off, while the harvested water of Harvesting RTC was posed to a risk of being mixed with newly entered stormwater. However, as a sacrifice, the total volume of water for harvesting collected from Harvesting-Environment RTC biofilters is much less than that collected from Harvesting RTC biofilters (5.4 % vs. 28.4 %) (Table 2).

3.4 Impact of RTC on TSS, TP and TN removal

We also wanted to ensure that RTC was not impacting greatly on our ability to maintain sediment and nutrient removal. The total load removal patterns for TSS, TN, and TP followed the E. coli removal patterns discussed above (Table 3). For each pollutant, the high load in the untreated stormwater that bypassed the Harvesting RTC resulted in a significant drop in load removal (compared to non-RTC) and the failure in meeting the requirements in Clause 56.07 in Victoria, Australia (State of Victoria, 2006) However, Harvesting-Environment RTC biofilters still met these requirements and had similar total removal rates as non-RTC biofilters, as all the inflow was treated. Furthermore, it is noted that, only three events were selected to test for TSS, TN, and TP, and the total removal rates might be different if all events were included.

Table 3 Total load removal rates of TSS, TP, and TN of RTC and non-RTC biofilters in the last three events (Event 9, Event 10, and Event 11) in each round of experiments.

		RTC biofilters	Non-RTC biofilters	Requirements in Clause 56.07 (State of Victoria, 2006)
Round 1	% total load removal of TSS	27 %	96 %	80 %
	% total load removal of TP	22 %	63 %	45 %
	% total load removal of TN	26 %	75 %	45 %
Round 2	% total load removal of TSS	97 %	97 %	80 %
	% total load removal of TP	82 %	78 %	45 %
	% total load removal of TN	77 %	78 %	45 %

The TSS removal rate of RTC-harvesting was comparable to that of non-RTC outflow in Event 9 (median dry event) (p = 0.777 for Round 1 and p = 0.337 for Round 2; one-way ANOVA), and was much lower than that of non-RTC outflow in Event 11 (long dry event) (p < 0.001; one-way ANOVA), indicating that long retention time could jeopardise TSS removal. That was because, due to the low moisture content, filter media might remobilise during long dry days and be drained out by the outflow in the next event (Table 4). Similar results were found in TP removal (Table 4), as the release of phosphorous could occur during extended dry weather periods (Bratieres et al., 2008). For TN removal, median dry period was preferred, as the TN removal rates of RTC-harvesting were significantly higher than those of non-RTC outflow in Event 9 in both rounds (p < 0.001 for Round 1 and p = 0.002 for Round 2; one-way ANOVA), while the benefit disappeared after long dry weather periods (Event 11 in Round 1) (p > 0.792; one-way ANOVA), due to the leaching of nitrogen (Bratieres et al., 2008; Payne et al., 2014) (Table 4). Therefore, both RTC strategies were effective for TN removal in harvesting, but neither were favoured in TSS or TP removal.

Table 4 TSS, TP and TN removal rates of RTC and non-RTC biofilters in the last three events (Event 9, Event 10, and Event 11) in each round. All the removal rates are concentration reductions. The cells were filled with "N/A" when no corresponding samples were collected.

		Event 9 (median dry: 5 days)			Event 10 (short dry: 1 day)			Event 11 (long dry: 21 days)		
Round 1	**Inflow concentration**	TSS: 160 mg/L; TP: 0.35 mg/L; TN: 1.7 mg/L			TSS: 190 mg/L; TP: 0.36 mg/L; TN: 1.7 mg/L			TSS: 150 mg/L; TP: 0.29 mg/L; TN: 1.5 mg/L		
		RTC biofilters		**Non-RTC biofilters**	**RTC biofilters**		**Non-RTC biofilters**	**RTC biofilters**		**Non-RTC biofilters**
		RTC-harvesting	RTC-environment (bypassed)	Non-RTC outflow	RTC-harvesting	RTC-environment (bypassed)	Non-RTC outflow	RTC-harvesting	RTC-environment (bypassed)	Non-RTC outflow
	Mean TSS removal rate (SD*)	97 % (1 %)	0 % (0 %)	97 % (1%)	N/A	0 % (0 %)	98 % (0 %)	72 % (14 %)	0 % (0 %)	91 % (1 %)
	Mean TP removal rate (SD)	68 % (10 %)	0 % (0 %)	60 % (11 %)	N/A	0 % (0 %)	58 % (11 %)	29 % (25 %)	0 % (0 %)	55% (11 %)
	Mean TN removal rate (SD)	87 % (2 %)	0 % (0 %)	76 % (4 %)	N/A	0 % (0 %)	84 % (4 %)	43 % (19 %)	0 % (0 %)	46 % (6 %)
Round 2	**Inflow concentration**	TSS: 170 mg/L; TP: 0.36 mg/L; TN: 2.2 mg/L			TSS: 180 mg/L; TP: 0.33 mg/L; TN: 2.2 mg/L			TSS: 195 mg/L; TP: 0.36 mg/L; TN: 2.3 mg/L		
		RTC biofilters		**Non-RTC biofilters**	**RTC biofilters**		**Non-RTC biofilters**	**RTC biofilters**		**Non-RTC biofilters**
		RTC-harvesting	RTC-environment (bypassed)	Non-RTC outflow	RTC-harvesting	RTC-environment (bypassed)	Non-RTC outflow	RTC-harvesting	RTC-environment (bypassed)	Non-RTC outflow
	Mean TSS removal rate (SD)	95 % (1 %)	98 % (0 %)	97 % (1 %)	N/A	99 % (0 %)	93 % (4 %)	N/A	99 % (0 %)	95 % (1 %)
	Mean TP removal rate (SD)	79 % (6 %)	81 % (6 %)	73 % (8 %)	N/A	84 % (5 %)	81 % (3 %)	N/A	72 % (9 %)	70 % (4 %)
	Mean TN removal rate (SD)	91 % (2 %)	86 % (2 %)	90 % (1 %)	N/A	94 % (1 %)	93 % (1 %)	N/A	54 % (8 %)	56 % (6 %)

*SD: Standard deviation.

4. Conclusion, implications to practical application, and future work

Both RTC strategies were effective in reducing the risks posed by faecal microbes during stormwater harvesting and reuse. Although RTC Strategy 2 (Harvesting-Environment) provided less volume of water for harvesting as compared to RTC Strategy 1 (Harvesting), it was able to (1) provide treated harvested water that meets both the requirement of dual reticulation with indoor and outdoor use or irrigation of commercial food crops (NHMRC, 2009) and the requirement for secondary contact recreation in Australia (NHMRC, 2008), and (2) discharge water to the environment which met Urban stormwater best practice environmental management guidelines (CSIRO, 1999)..

This study was the first attempt to apply RTC in stormwater biofilters and demonstrated that RTC systems can deliver safe water for harvesting and reuse. The two strategies developed in this study are easy and cheap to be implemented both in newly developed systems and for the retrofit of existing systems. While RTC biofilters are promising for practical applications, several points are worthwhile to note:

- The set points of the two strategies used in this experiment (e.g., minimum required retention time T_{min} = 2 days) might be different when the strategies are applied in another biofilter and/or with different climate conditions and inflow features. The set points may need to be adjusted in newly developed RTC strategies.
- To increase the harvested water volume in RTC Strategy 2 (Harvesting-Environment), a possible method in practice could be to enlarge the submerged zone (SZ) volume of biofilters during the design process. A larger SZ could also supply plants with more water to resist extended dry weather periods. The cost-effectiveness of enlarging SZ volumes will be evaluated in future work.
- During the implementation of RTC Strategy 1 (Harvesting), the maximum volume of stormwater that entered biofilters in a single event was the submerged zone volume; however, we underestimated the water holding capacity of the soil and suggest that additional stormwater

could be allowed to enter biofilters without causing any discharge of newly entered water.

- We know that extended retention time (T_{min}) is crucial for microbial (and nutrient) removal, but we suggest that a maximum retention time (T_{max}) could help mitigating the impact of extended dry period and balancing the removal of microbes and nutrients, as we observed that the leaching of TN and the release of TP occurred during extended dry period.
- Finally, no historical rainfall forecast archive was available in this study, and future work will be conducted to explore the potential uncertainties caused by inaccurate rainfall forecast. It is expected that the unreliability of rainfall forecast could negatively impact the effectiveness of RTC.

Acknowledgement

The authors would like to thank Richard Williamson, Anthony Brosinsky, Christelle Schang, Harsha Fowdar, Mohammed Al-Ameri, Juri Jung, Veljko Prodanovic, Wenjun Feng, Natalie Barron and other team members from EPHM Lab and Monash University for their assistance in columns set-up and experiments implemenation.

References

APHA-AWWA-WEF. Standard Methods for the Examination of Water and Wastewater, the 21st. Edition: American Public Health Association, Washington, 2005.

Bratieres K., Fletcher T. D., Deletic A., Zinger Y. Nutrient and sediment removal by stormwater biofilters: A large-scale design optimisation study. Water Research 2008; 42: 3930-3940.

Chandrasena G. I. Faecal Microorganism Removal in Stormwater Biofilters. Department of Civil Engineering. Doctor of Philosophy. Monash University, 2014.

Chandrasena G. I., Deletic A., A. L., Henry R., McCarthy D. T. Stormwater biofilters as barriers against *Campylobacter jejuni*, *Cryptosporidium*

oocysts and adenoviruses; results from a laboratory trial. Water 2017; 9: 949.

Chandrasena G. I., Deletic A., Ellerton J., McCarthy D. T. Evaluating *Escherichia coli* removal performance in stormwater biofilters: a laboratory-scale study. Water Science & Technology 2012; 66: 1132-1138.

Chandrasena G. I., Deletic A., McCarthy D. T. Survival of *Escherichia coli* in stormwater biofilters. Environmental Science and Pollution Research 2014a; 21: 391-401.

Chandrasena G. I., Deletic A., McCarthy D. T. Biofiltration for stormwater harvesting: Comparison of *Campylobacter spp.* and *Escherichia coli* removal under normal and challenging operational conditions. Journal of Hydrology 2016; 537: 248-259.

Chandrasena G. I., Pham T., Payne E. G., Deletic A., McCarthy D. T. *E. coli* removal in laboratory scale stormwater biofilters: Influence of vegetation and submerged zone. Journal of Hydrology 2014b; 519: 814-822.

Czemiel Berndtsson J. Green roof performance towards management of runoff water quantity and quality: A review. Ecological Engineering 2010; 36: 351-360.

Davies C. M., Bavor H. J. The fate of stormwater associated bacteria in constructed wetland and water pollution control pond systems. Journal of Applied Microbiology 2000; 89: 349-360.

Duncan H. P. Urban Stormwater Quality: A Statistical Overview. Melbourne, Australia: Cooperative Research Centre for Catchment Hydrology, 1999.

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

Fletcher T. D., Deletic A., Mitchell V. G., Hatt B. Reuse of urban runoff in Australia: a review of recent advances and remaining challenges. Journal of Environmental Quality 2008; 37: 116 - 127.

Fowdar H. S., Hatt B. E., Breen P., Cook P. L. M., Deletic A. Designing living walls for greywater treatment. Water Research 2017; 110: 218-232.

Hathaway J., Hhunt W., Graves A., Wright J. Field Evaluation of Bioretention Indicator Bacteria Sequestration in Wilmington, North Carolina. Journal of Environmental Engineering 2011; 137: 1103-1113.

Hathaway J., Hunt W. Indicator Bacteria Removal by Stormwater BMPs in Coastal North Carolina. World Environmental and Water Resources Congress: Challenges of Change, Providence, Rhode Island, United States, 2010, pp. 3218-3225.

Hatt B. E., Fletcher T. D., Deletic A. Hydrologic and pollutant removal performance of stormwater biofiltration systems at the field scale. Journal of Hydrology 2009; 365: 310-321.

Hsu N.-S., Huang C.-L., Wei C.-C. Multi-phase intelligent decision model for reservoir real-time flood control during typhoons. Journal of Hydrology 2015; 522: 11-34.

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

Jung J., Fowdar H., Henry R., Deletic A., McCarthy D. Biofilters as effective pathogen barriers for greywater reuse. Ecological Engineering 2019: under review.

Kirk R. E. Introduction to the Analysis of Variance. Statistics: An Introduction (5th edition). Thomson/Wadsworth, Belmont, CA, 2008, pp. 392-429.

Leon A. S., Kanashiro E. A., Valverde R., Sridhar V. Dynamic Framework for Intelligent Control of River Flooding: Case Study. Journal of Water Resources Planning and Management 2014; 140: 258-268.

Li Y., McCarthy D. T., Deletic A. *Escherichia coli* removal in copper-zeolite-integrated stormwater biofilters: Effect of vegetation, operational time, intermittent drying weather. Ecological Engineering 2016; 90: 234-243.

Li Y. L., Deletic A., Alcazar L., Bratieres K., Fletcher T. D., McCarthy D. T. Removal of Clostridium perfringens, Escherichia coli and F-RNA coliphages by stormwater biofilters. Ecological Engineering 2012; 49: 137-145.

McCarthy D. T., Deletic A., Mitchell V. G., Fletchera T. D., Diaper C. Uncertainties in stormwater E. coli levels. Water Research 2008; 42: 1812-1824.

Meng Z., Chandrasena G., Henry R., Deletica A., Kolotelo P., McCarthy D. Stormwater constructed wetlands: A source or a sink of *Campylobacter* spp. Water Research 2018; 131: 218-227.

Mitchell V. G., Deletic A., Fletcher T. D., Hatt B. E., McCarthy D. T. Achieving multiple benefits from stormwater harvesting. Water Science & Technology 2007; 55: 135-144.

Murphy H. M., Meng Z., Henry R., Deletic A., McCarthy D. T. Current Stormwater Harvesting Guidelines Are Inadequate for Mitigating Risk from *Campylobacter* During Nonpotable Reuse Activities. Environmental science & technology 2017; 51: 12498-12507.

NHMRC. Guidelines for Managing Risks in Recreational Water. Australian Government, National Health and Medical Research Council, Canberra, Australia, 2008.

NHMRC. Australian Guidelines for Water Recycling (Phase 2). Stormwater Harvesting and Reuse. Australian Government, Natural Resource Management Ministerial Council, Environment Protection and Heritage Council, National Health and Medical Research Council, Canberra, Australia, 2009.

Payne E. G. I., Pham T., Cook P. L. M., Fletcher T. D., Hatt B. E., Deletic A. Biofilter design for effective nitrogen removal from stormwater – influence of plant species, inflow hydrology and use of a saturated zone. Water Science & Technology 2014; 69: 1312-1319.

Schilling W., Andersson B., Nyberg U., Aspegren H., Rauch W., Harremoës P. Real time control of wastewater systems. Journal of Hydraulic Research 1996; 34: 785-797.

Schütze M., Campisano A., Colas H., Schilling W., Vanrolleghem P. A. Real time control of urban wastewater systems—where do we stand today? Journal of Hydrology 2004; 299: 335-348.

State of Victoria D. o. S. a. E., Australia. Using the integrated water management provisions of Clause 56 - Residential subdivision, Victoria, Australia, 2006.

Stevik T. K., Aa K., Ausland G., Hanssen J. F. Retention and removal of pathogenic bacteria in wastewater percolating through porous media: a review. Water Research 2004; 38: 1355-1367.

Zhang L., Seagren E. A., Davis A. P., Karns J. S. The Capture and Destruction of *Escherichia coli* from Simulated Urban Runoff Using Conventional

Bioretention Media and Iron Oxide-coated Sand. Water Environment Research 2010; 82: 701-714.

Zhang L., Seagren E. A., Davis A. P., Karns J. S. Long-term sustainability of escherichia coli removal in conventional bioretention media. Journal of Environmental Engineering 2011; 137: 669-677.

Zinger Y., Deletic A., Fletcher T. D., Breen P., Wong T. A Dual-mode Biofilter System: Case study in Kfar Sava, Israel. 12th International Conference on Urban Drainage, Porto Alegre, Brazil, 2011.

Supplementary material

We propose that the improved removal rates observed in the RTC columns (compared to removal rates observed in the non-RTC columns) in in Round 1 and Round 2 (Section 3.1.1 and 3.1.2) were both mainly derived from the implementation of RTC strategies, rather than the inherent differences between biofilters (i.e., RTC vs. non-RTC, and with vs. without woodchips). This was because: (1) at the start of each round, there was no significant difference between the infiltration rates of RTC biofilters and those of non-RTC biofilters (p = 0.518, two-way ANOVA; Round and biofilter type); and (2) compared to the type of biofilters, time was a much more significant factor to the change of infiltration rates through the experiments (with much lower p value and p < 0.001 in three-way ANOVA: Round, biofilter type, and time). The infiltration rates in each event are provided in Figure A.1.

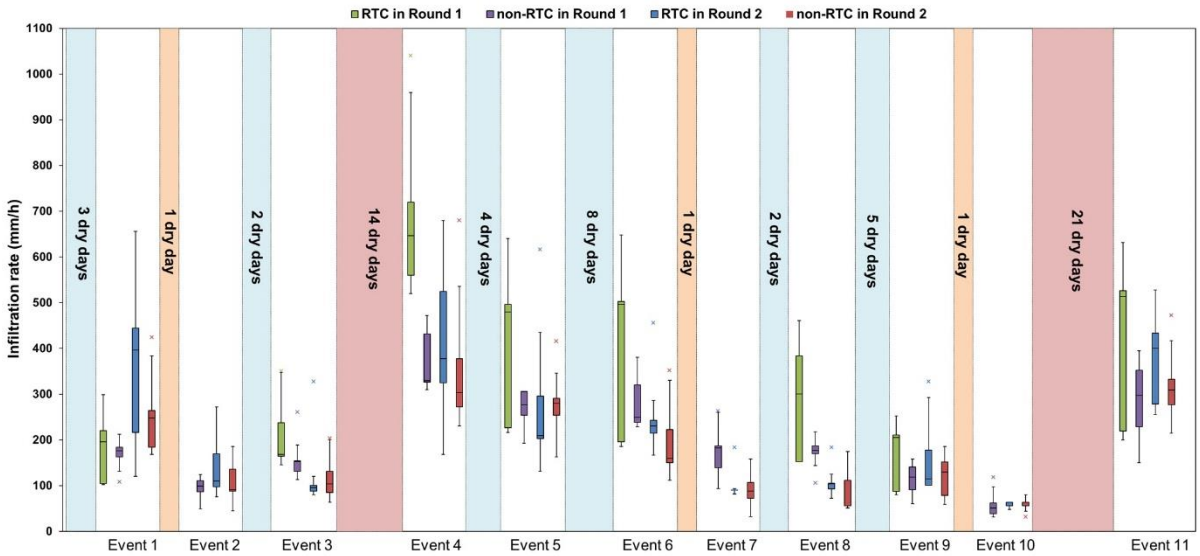


Figure A.1 Infiltration rates of RTC and non-RTC biofilters in each event of Round 1 and Round 2.

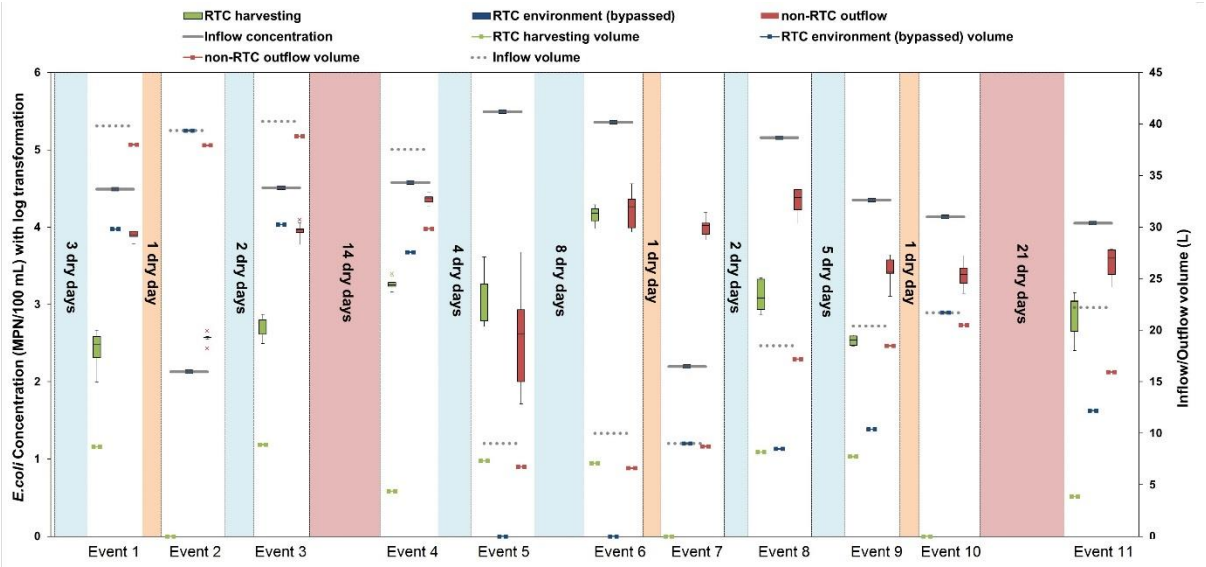


Figure A.2 Experimental results during the implementation of Harvesting RTC. In each event, a comparison between the *E. coli* concentrations (log transformed) of RTC-harvesting, RTC-environment (bypassed) and non-RTC outflow is included.

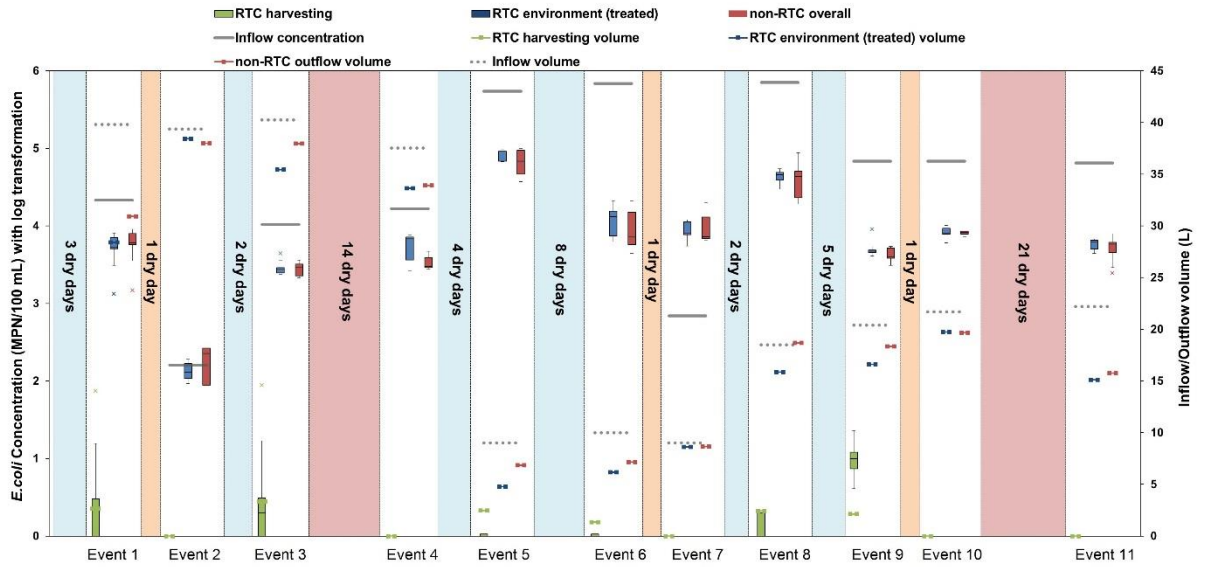


Figure A.3 Experimental results during the implementation of Harvesting-Environment RTC. In each event, a comparison between the *E. coli* concentrations (log transformed) of RTC-harvesting, RTC-environment (bypassed) and non-RTC outflow is included.

5.3 Discussion and conclusions

This study presents the first attempt to develop and test RTC in stormwater biofilters for the purpose of stormwater harvesting and reuse. The developed two RTC strategies, Harvesting RTC and Harvesting-Environment RTC, focus on different priorities and could both provide benefits for stormwater harvesting by improving the quality of supplied water. Therefore, it is concluded that, adopting RTC in stormwater biofilters is feasible and effective to eliminate the risks posed by faecal microbes during stormwater harvesting and reuse.

In addition, it is expected that the additional information (e.g., water level and moisture content) monitored by RTC equipment (e.g., flowmeters and sensors) could lead to a more effective maintenance of RTC biofilters compared to non-RTC biofilters, so that the performance of the entire system would be improved. For example, the occurrence of clogging could be detected by a moisture sensor, and prompt maintenance could be conducted based on this information.

In the meantime, the results of laboratory experiments indicated that neither of the developed strategies is perfect, as both of them have some limitations: e.g., Harvesting RTC exacerbated the pollution to the environment as untreated stormwater with a large amount of load was bypassed, while Harvesting-Environment RTC only collected limited volume of stormwater due to soil holding capacity during the free drainage of the harvested water. These results indicated that, although it is aimed to collect water with good quality and large volume, how to balance these competing needs during RTC implementation is still a question.

To solve this problem, one option would be developing and testing additional strategies, and selecting strategies according to the strategy effectiveness, end use requirements, and treatment priorities. To achieve this aim, it would be very time-consuming and not economically feasible to test all the potential strategies one by one through laboratory experiments or field tests; instead, modelling might be an efficient tool to assess RTC strategies, as long as the selected model is proved to be effective in reflecting the outcome of RTC implementation.

The model developed in Chapter 3 would be a potential option, however, it was only developed for non-RTC biofilters and therefore needs to be modified and validated with the data collected from RTC experiments. This work will be conducted in the next chapter (Chapter 6).

Furthermore, in this study, only laboratory experiments were conducted in RTC testing, as under laboratory conditions, it is easy to fulfil system control and monitoring, and the test is repeatable. However, the outcome of RTC implementation in a field system (where extreme weather conditions and larger system scale may apply) is still unknown. Considering the difficulty to conduct and the high uncertainty in field monitoring and control, again, modelling could also be adapted to evaluate the performance of RTC strategies with a modelled representation of reality. This work will also be presented in the next chapter.

5.4 References

Chandrasena G. I., Deletic A., Ellerton J., McCarthy D. T. Evaluating *Escherichia coli* removal performance in stormwater biofilters: a laboratory-scale study. Water Science & Technology 2012; 66: 1132-1138.

Chandrasena G. I., Pham T., Payne E. G., Deletic A., McCarthy D. T. *E. coli* removal in laboratory scale stormwater biofilters: Influence of vegetation and submerged zone. Journal of Hydrology 2014; 519: 814-822.

Li Y. L., Deletic A., Alcazar L., Bratieres K., Fletcher T. D., McCarthy D. T. Removal of Clostridium perfringens, Escherichia coli and F-RNA coliphages by stormwater biofilters. Ecological Engineering 2012; 49: 137-145.

Schütze M., Butler D., Beck B. M. Modelling, Simulation and Control of Urban Wastewater Systems: Springer Science & Business Media, 2002.

Chapter 6.

Model modification and validation for real time control, and further exploration of control strategies

6.1 Introduction

The two developed and tested RTC strategies in Chapter 5, Harvesting RTC and Harvesting-Environment RTC, have been proved effective to reduce the risks posed by faecal microbes during stormwater harvesting and reuse, as harvestable water has been collected. However, limitations still exist in both strategies. For example, a large amount of untreated stormwater is bypassed by Harvesting RTC and exacerbates environmental pollution, while limited volume of harvested water was collected from Harvesting-Environment RTC biofilters, due to the soil holding capacity. Indeed, competing needs (e.g., larger amount of water and better water quality) may exist during harvesting. To balance these needs, more RTC strategies need to be tested.

However, it is very time-consuming and not economically feasible to test all the potential RTC strategies one by one through laboratory or field testing. Instead, modifying the model developed in Chapter 3 might be effective to generate an effective tool for RTC evaluation. Therefore, the first objective of this study is to analyse the feasibility of modifying the model that developed in Chapter 3 to simulate the outcome of RTC implementation.

If the modified model is capable of representing the benefits of RTC through simulation, it could be used to explore additional RTC strategies. In addition, considering that the effectiveness of RTC with field inputs is still unknown, modelling could also be employed to assess the RTC performance under the scenarios where the reality could be well represented (e.g., use modelling to represent the reality).

As such, the objectives of the study presented in this chapter are: (1) modify the developed model in Chapter 3 and evaluate the capability of the modified model in representing the benefits of RTC; (2) if the modified model is capable of representing the benefits of RTC implementation, utilise the model to explore and assess more strategies and scenarios, exploring more RTC options to balance the competing needs that exist in stormwater harvesting and reuse. The research questions include:

- Is the outcome of RTC implementation could be well simulated by the model developed for non-RTC bioifilters after modification, with the parameters calibrated for non-RTC bioifilters?
- Could the competing needs in stormwater harvesting and reuse be balanced by optimising developed RTC strategies or developing new RTC strategies?
- What are the differences of operational schemes and outcomes when a same RTC strategy is applied in different biofilter systems and/or with different inputs?

Corresponding to the research questions, two hypotheses were made:

- The model that developed for non-RTC bioifilters is capable of representing the outcome of RTC implementation after model modification, since the model is proved to be effective in simulating various operational conditions, and hence the change of operational conditions that triggered by RTC is expected to be well reflected; the only modification needed is to enable the model to represent potential control behaviors (e.g., suffocating inflow/outflow pipes);
- The optimisation of developed RTC and the development of new RTC could be effective in balancing the competing needs in stormwater harvesting and reuse; however, the outcome may also depend on the inputs (e.g., rainfall characteristics, and inflow concentrations);
- For different systems with different inputs, when a same RTC strategy is applied, the optimum set-points and corresponding benefits may vary, depends on the inputs and treatment requirement.

This chapter presents the study and results of modifying the model developed in Chapter 3 and using the modified model to simulate the laboratory experiments that conducted in Chapter 5. In addition, two additional RTC strategies are assessed in the modelling scenarios using a modelled representation of reality. This chapter is written as a draft for journal publication and will be submitted to *Journal of Hydrology*.

6.2 Model validation and further exploration of real time control

BioRTC: a new model that simulates and explores real time control strategies of stormwater biofilters

Pengfei Shen [a]*, Ana Deletic [b] David T. McCarthy [a]

[a] Environmental and Public Health Microbiology Laboratory (EPHM Lab), Department of Civil Engineering, Monash University, Clayton, VIC 3800, Melbourne, Australia

[b] School of Civil and Environmental Engineering, University of New South Wales, Sydney, NSW 2052, Australia

* Corresponding author. Email address: [redacted]

Abstract

This study uses modelling to explore real time control (RTC) strategies for biofilters so that they can be used to treat stormwater and deliver harvestable stormwater. To achieve this aim, a model for the prediction of microbial removal in stormwater biofilters was first selected, modified to include RTC capabilities, and validated with data collected from a set of laboratory experiments. These experiments trialled two real time control strategies of biofilters, both focusing on simple rules to harvest well-treated submerged zone water. After modification, the new BioRTC model was able to represent the behaviour of the laboratory biofilters and the RTC operations well, with Nash Sutcliffe Efficiencies (E_c) of up to 0.80. This validated model was then used to explore the potential of an additional two new RTC strategies. The first strategy retains water in biofilters for a sufficient time to ensure optimal die-off before allowing any further inputs into the system, and when inputs are allowed to enter, the input volume is restricted to the submerged zone's pore volume plus the unsaturated zone's water holding capacity. The second strategy enlarges biofilters' submerged zone depth to 1.5 times greater, uses a bottom pipe to collect the water that has sufficiently treated by biofilters before the start of next

rainfall event for harvesting, and closes the bottom pipe and operates biofilters as normal when stormwater enters. Compared to the previously developed RTC strategies, the two new strategies could better protect the environment by removing higher pollutant loads. With these simple modifications, they could also better balance the competing needs in stormwater harvesting and reuse, in that they could achieve a higher volume of harvestable water together with comparable or improved water quality. Moreover, by analysing the Pareto fronts under different set-point values, the optimum set-point for each strategy in the tested scenarios were recommended. The results also indicated that different RTC designs and set-points are needed for each new application, and BioRTC could be an effective tool to implement this suggestion.

Keywords: stormwater biofilters, real time control, modelling, stormwater harvesting, microbes, *E. coli*

1. Introduction

Faecal microbes are the major pollutants that prevent stormwater harvesting and reuse, as they pose serious risks to human health (Meng et al., 2018; Murphy et al., 2017). As such stormwater needs to be treated before harvesting. To treat stormwater, biofilters (also named as bioretention systems or rain gardens) have been widely adopted all over the world (Bratieres et al., 2008; Hathaway et al., 2011; Hatt et al., 2009; Zinger et al., 2011). Stormwater biofilters are soil-plant systems, using enhanced infiltration and evapotranspiration to eliminate faecal microbes and other pollutants (e.g., nutrients and heavy metals) (FAWB, 2009). For microbial removal in stormwater biofilters, adsorption, desorption, straining and die-off are the governing processes (Chandrasena et al., 2014a; Stevik et al., 2004).

Although stormwater biofilters are more effective in faecal microbial removal compared to other water sensitive urban design systems (e.g., wetlands) (Chandrasena et al., 2012; Davies and Bavor, 2000; Hathaway and Hunt, 2010), further optimisation is needed, as their performance was reported to be inconsistent. For example, the removal rates for *E. coli* (*Escherichia coli*) and *Campylobacter* spp. could both range from > 99 % to < 0 % (Chandrasena et

al., 2016; Chandrasena et al., 2014b; Li et al., 2012; Zhang et al., 2011; Zhang et al., 2012). The major reason for this high variability is, the negative effects that result from various operational conditions could not be mitigated by design optimisation. These operational conditions include extended dry periods (cause plant death and preferential flow), short dry periods (i.e. insufficient die-off of trapped organisms), and big size events (cause the breakthrough of treatment capacity) (Chandrasena et al., 2012; Chandrasena et al., 2014b; Li et al., 2012). To further enhance microbial removal in biofilters to achieve better water quality for stormwater harvesting, operational conditions need to be controlled.

To achieve control and optimisation of stormwater biofilters, real time control (RTC) is a potential tool. The general concept of RTC is monitoring a system in real time, and using collected data from monitoring to optimise operation by controlling certain aspects of the system (Schütze et al., 2004). RTC has been widely adopted in flooding control, and sewer systems and wastewater treatment optimisation (Hsu et al., 2015; Leon et al., 2014; Schilling et al., 1996; Schütze et al., 2004). Recently, the first attempt of applying RTC in stormwater biofilters has been carried out by Shen et al. (2018a) by developing two RTC strategies and testing them via laboratory experiments. The first RTC strategy focuses on delivering the best water quality for harvesting and reuse or for recreational waterways, by restricting the input volume to the submerged zone's pore volume. Therefore, a part of untreated stormwater was bypassed and the environment was polluted. While the second RTC strategy builds upon the first strategy and focuses on delivering a balance between good water quality for harvesting and protecting the environment. However, only a very limited volume of stormwater was collected (Shen et al., 2018a). The experimental testing results indicated that, although the two developed RTC strategies were both effective in reducing the risks posed by faecal microbes during stormwater harvesting and reuse, some competing needs (e.g., good water quality and large water volume for harvesting) could not be well balanced.

To balance the competing interests of using biofilters to deliver water with harvestable quality and sufficient volume, while at the same time protecting the receiving waterbody, further RTC strategies need to be conceptualised and

tested. However, it is very time-consuming and not economically feasible to test all the potential RTC strategies and scenarios one by one through laboratory experiments or field tests, as in those conducted by Shen et al. (2018a). Instead, modelling could be considered as a potential tool to efficiently evaluate RTC strategies and scenarios.

This study therefore aims to analyse the feasibility of using modelling to simulate the results of RTC implementation and assess further options of RTC to balance the competing needs in stormwater harvesting and reuse. To achieve this aim, a new model BioRTC was developed based on the modification of an existing model to include real time control capabilities. BioRTC was then validated using the published datasets of Shen et al. (2018a), which tested RTC strategies on laboratory biofilter columns. In addition, two new RTC strategies were conceptualised and tested using the BioRTC model.

2. Methods

To achieve this aim, we follow this framework: Section 2.1, select an appropriate model that can represent microbial removal in stormwater biofilters; Section 2.2, modify the model to include real time control capabilities and validate this new model (BioRTC) can represent existing studies that have monitored the performance of RTC on stormwater biofilters; Section 2.3, conceptualise new RTC strategies; and, Section 2.4, test these strategies using the validated BioRTC model.

2.1 Model selection

Only a limited number of models have been developed for microbial removal prediction in stormwater biofilters (Chandrasena et al., 2013; Shen et al., 2018b; Zhang et al., 2010; Zhang et al., 2012). Among them, the process-based model developed by Shen et al. (2018b) is promising, as it includes the major processes known to influence microbial removal (adsorption, desorption, and die-off) and the primary factor associated with microbial die-off (temperature). Furthermore, it has demonstrated capabilities of predicting the performance of different laboratory-scale and field-scale biofilters (Shen et al., 2018b; Shen et al., 2018c). However, the model does not include RTC capabilities, nor has it

been applied or tested using data from biofilters that have RTCs. As such, we first provide an overview of the model and its structure, and later discuss how it has been amended to include RTC capabilities.

The model details have been fully described by Shen et al. (2018b) and hence it is briefly summarised. The model utilises a “three-bucket” approach and these buckets represent the major parts of a typical stormwater biofilter: (1) a ponding zone (i.e., the temporary pond appears on the top of filter media when inflow comes), (2) an unsaturated zone (USZ; i.e., the part of filter media where is not saturated), and (3) a submerged zone (SZ; i.e., the part of filter media where is saturated; normally created by raising the outflow pipe). The model has two modules, the flow module and microbial quality module.

The flow module simulates the governing flow processes in biofilters. For example, Darcy’s law is adopted to describe infiltration (Dingman, 2002), while the functions introduced in FAO-56 are employed to represent evapotranspiration (Allen et al., 1998). This module has two parameters: (1) K_s - hydraulic conductivity of biofilters, and (2) K_c - plant coefficient for evapotranspiration, to reflect the water uptake ability of the tested plants. The key equations in the flow module are listed in Table 1, where h_p, h_{usz}, and h_{sz} are respectively the depths of ponding zone, USZ, and SZ, S is the saturation level of USZ, n_{usz} and n_{sz} are respectively the porosity in USZ and SZ, Q_{in} is the inflow of biofilters, A is the biofilter surface area, Q_{over} is the overflow of biofilters, Q_{hc} is the capillary rise from SZ to USZ, ET_0 is the potential evapotranspiration rate, S_{entire} is the entire saturation level of USZ plus SZ, S_w and S_s are the thresholds of saturation level for different stages of evapotranspiration, S_{entire} is the field capacity of filter media, γ is the hydraulic conductivity coefficient.

Table 1 Key equations in the flow module of the model presented by Shen et al. (2018b).

Flow module equation	Eq. No.
General form of equations	
Flow = min (physically possible; available upstream; available downstream)	
Ponding zone (PZ)	
Infiltration from PZ to unsaturated zone	
$Q_{pf} = \min\left(K_s A \frac{h_p + h_{usz}}{h_{usz}}, \frac{h_p A_p}{dt} + Q_{in}, \frac{1}{dt}(1-S) n_{usz} h_{usz} A\right)$	(1)
Water mass balance in PZ	
$\frac{d(h_p A)}{dt} = Q_{in} - Q_{pf} - Q_{over}$	(2)
Unsaturated zone and saturated zone (USZ and SZ)	
Total Evapotranspiration from USZ and SZ	
$Q_{et} = \begin{cases} 0, S_{entire} \le S_w \\ A \times K_c \times ET_0 \frac{S_{entire} - S_w}{S_s - S_w}, S_w < S_{entire} \le S_s \\ A \times K_c \times ET_0, S_s < S_{entire} \le 1 \end{cases}$	(3)
Unsaturated zone (USZ)	
Evapotranspiration from USZ	
$Q_{et\_usz} = Q_{et} \times \frac{S \times n_{usz} h_{usz}}{S \times n_{usz} h_{usz} + n_{sz} h_{sz}}$	(4)
Infiltration from USZ to SZ	
$Q_{fs} = \begin{cases} \min\left(A \times K_s \frac{h_p + h_{usz}}{h_{usz}} S^{\gamma}, \frac{(S - S_{fc}) A \times n_{usz} h_{usz}}{dt} + Q_{pf} + Q_{hc}\right), S \ge S_{fc} \\ 0, S < S_{fc} \end{cases}$	(5)
Water mass balance in USZ	
$\frac{d(S \times n_{usz} h_{usz} A)}{dt} = Q_{pf} + Q_{hc} - Q_{fs} - Q_{et\_usz}$	(6)
Saturated zone (SZ)	
Evapotranspiration from SZ	
$Q_{et\_sz} = Q_{et} - Q_{et\_usz}$	(7)
Flow through drainage pipe	
$Q_{pipe} = \begin{cases} \min\left(A \times K_s \frac{h_p + h_{usz}}{h_{usz} + h_{sz}}, \frac{(h_{sz} - h_{pipe}) n_{sz} A}{dt} + Q_{fs} - Q_{hc} - Q_{et\_sz}\right), h_{sz} > h_{pipe} \\ 0, h_{sz} \le h_{pipe} \end{cases}$	(8)

The microbial quality module predicts the behaviour of microbes using one-dimensional advection-dispersion equations, with the flow information obtained

from the flow module. The water quality module has four parameters: adsorption rate (k_{att}), desorption rate (k_{det}), the standard die-off rate (μ_0), and the temperature coefficient for die-off (θ). Take USZ for example, the key equations in this module were listed in Table 2, where c_{usz}: the microbial concentration in USZ, ρ: bulk soil density, M_1: the the microbial concentration in the solid phase due to adsorption, D_1: the dispersion coefficient in USZ, and q_1: average unit flow in USZ. Detailed equations and parameters are provided in Supplementary material A, for all zones and both the water quality and flow modules.

Table 2 Key equations for the unsaturated zone in the microbial quality module of the model presented by Shen et al. (2018b).

Microbial quality module key equations in USZ	Eq. No.
Microbial mass balance in the water phase	
$\frac{\partial(Sn_{usz}c_{usz})}{\partial t} + (Sn_{usz}k_{att}c_{usz} - \rho k_{det}M_1) = \frac{\partial}{\partial z}\left(Sn_{usz}D_1\frac{\partial c_{usz}}{\partial z}\right) - \frac{\partial(q_1c_{usz})}{\partial z} - Sn_{usz}\mu c_{usz}$	(9)
Adsorption, desorption and die-off of adsorbed microbes in the soil phase	
$\frac{\partial M_1}{\partial t} = \frac{n_{usz}S}{\rho}k_{att}c_{usz} - k_{det}M_1 - \mu M_1$	(10)
Die-off rate in USZ	
$\mu = \mu_0\theta^{T-20℃}$	(11)

2.2 Model modification and validation

In this section we describe the RTC dataset used for model validation (Section 2.2.1), the modification of the existing model introduced in Section 2.1 to incorporate RTC capabilities (Section 2.2.2), and the modelling process employed to conduct this validation (Section 2.2.3).

2.2.1 Summary of dataset used for validation

Harvesting RTC is the first strategy tested by Shen et al. (2018a). It is a strategy that retains water in the biofilter for an adequate time to ensure optimal die-off before allowing any further stormwater into the system. In addition, when

stormwater is allowed to enter, the input volume is restricted to the submerged zone's pore volume, and the rest of stormwater is bypassed. Therefore, although this RTC collects "clean" stormwater that has sufficient retention time, it would pollute the environment by bypassing significant amounts of untreated stormwater. To implement Harvesting RTC, a flowmeter to detect the inflow volume (V_{in}), an inflow control valve to open or close the inflow pipe, and a timer to monitor the retention time of retained stormwater in biofilters (T) are needed. The major control processes are: (1) when stormwater inflow is detected, if $T < T_{min}$ (minimum required retention time), keep the inflow control valve closed and bypass all influent; (2) if $T \geq T_{min}$, open the inflow control valve, and record the flow/volume of the stormwater entering the biofilter (V_{in}); and, (3) when $V_{in} = V_{sz}$, close the inflow control valve and bypass the rest of stormwater to the environment. These processes ensure that all the retained water with sufficient retention time is collected, and the submerged zone pore volume is filled with newly entered stormwater. The schematic of Harvesting RTC rules could be found in Supplementary material B (Figure B.1).

Harvesting-Environment RTC is the second strategy tested by Shen et al. (2018a). It has similar concepts as the previous strategy, but is slightly more difficult to implement but the benefits are that less water is bypassed around the system. In addition to the raised outflow pipe typically found in biofilters, a bottom pipe that at the base of biofilter is required. Three hours before next rainfall event begins, the water that has retained in biofilters for sufficient is drained and collected for harvesting through the bottom pipe. When stormwater inflow begins, the bottom pipe is closed, and the biofilter operates as normal and drains via a raised outlet pipe, discharging the treated stormwater to the environment. To implement this RTC, rainfall forecast information, a timer to record the retention time of retained stormwater in biofilters (T), and a bottom pipe with a control value are needed. The major control processes are: (1) when the next wet event is around three hours away (according to rainfall forecast), if $T < T_{min}$ (minimum required retention time), keep the bottom pipe closed using the control valve, and biofilters will be operated as normal in the coming event; (2) if $T \geq T_{min}$, open the bottom pipe and collect SZ water via the bottom pipe; and (3) when stormwater inflow is detected, close the bottom pipe and operate

biofilters as normal again. The schematic of Harvesting RTC rules is provided in Supplementary material B (Figure B.1).

Laboratory experiments for RTC testing were done for both of these strategies, as detailed in Shen et al. (2018a). In brief, ten biofilter columns were established in a greenhouse at Monash University, Australia. They were divided into RTC biofilters (control was implemented) and non-RTC biofilters (without control), with five replicates each. All these columns had identical size, were planted with *Carex appressa*, and had washed sand as filter media. The size of each part of columns were: ponding depth - 280 mm, unsaturated zone depth - 500 mm, submerged zone depth - 440 mm, column diameter - 240 mm, biofilter's pore volume - 20 L, submerged zone pore volume - 10 L. The schematic of a tested column is shown in Supplementary material B (Figure B.3). Two rounds of experiments were conducted: Harvesting RTC was implemented in Round 1, while Harvesting-Environment RTC was conducted on the same columns during a second round, Round 2. In each round, 11 sampling events were designed and carried out based on Melbourne's historical rainfall. These events were with various rainfall sizes, antecedent dry lengths, and inflow concentrations. However, importantly, the characteristics of the *n*th Event in Round 1 was same as those of the *n*th Event in Round 2. The minimum required retention time of the SZ water (T_{min}) was set as two days, according to the findings by Chandrasena (2014). Biofilters were dosed with semi-natural stormwater, which was prepared by collecting the sediments from a local stormwater wetland inlet and mixing them with dechlorinated tap water, supplementing with laboratory grade chemicals, and spiking *Escherichia coli* (*E. coli*) to target the typical concentrations of different pollutants in stormwater (Duncan, 1999; McCarthy et al., 2008; NHMRC, 2009). During each event, inflow and outflow samples were collected and analysed for *E. coli* concentrations using the Colilert method™ (IDEXX-Laboratories, 2007). To help estimate infiltration rates, the instantaneous water depths in biofilters' ponding zone were recorded at regular intervals (3.75 minutes). To help understand evapotranspiration rates, total outflow volumes were measured after each dosing event.

2.2.2 Model modification: creation of BioRTC

To modify the selected model that described in Section 2.1 to suit the RTC capabilities used in the RTC laboratory experiments (introduced in Section 2.1.1) and other control potentials for biofilters, some components need to be added/adjusted in the equations of the flow module. As such, a new model, BioRTC, was developed. Compared to the original model, four modification patterns are provided (as follows), and the modified equations/parameters are also corresponding to the most typical operational conditions that are potentially adjustable:

a) Inflow equation modification

The inflow rate of a RTC system may need to be controlled (e.g., by a valve) to adjust the amount of water that enters biofilters (e.g., for Harvesting RTC), or to control the infiltration rate. Therefore, an equation for the inflow of biofilters, Q_{in}, needs to be added to the flow module in Table 1:

$$Q_{in} = \begin{cases} Q_{sw}, \text{ no control} \\ 0, \text{inflow pipe is fully closed} \\ Q_{in\_control}, \text{inflow is partly closed} \end{cases} \quad \text{Eq. (12)}$$

Where Q_{sw} is the stormwater inflow, $Q_{in\_control}$ is the controlled inflow (typically, the value could be determined by a flowmeter in RTC).

b) Outflow equation modification

Similar to the inflow, the outflow of a biofilter could also be controlled in a RTC system to adjust the retention time (e.g., for Harvesting-Environment RTC) or infiltration rate. Therefore, the Eq. (11) in Table 1 could be revised as:

$$Q_{pipe} = \begin{cases} \min\left(A \times K_s \frac{h_p + h_{usz}}{h_{usz} + h_{sz}}, \frac{(h_{sz} - h_{pipe}) n_{sz} A}{dt} + Q_{fs} - Q_{hc} - Q_{et\_sz}\right), h_{sz} > h_{pipe} \text{ and no control} \\ 0, h_{sz} \leq h_{pipe} \text{ or outflow pipe is fully closed} \\ A \times Cd\left(\frac{1}{4}\pi D^2\right)\sqrt{2gh}, \text{ outflow pipe is partly closed} \end{cases}$$

Eq. (13)

Where Q_{sw} is the stormwater inflow, *Cd* is the orifice discharge coefficient, D is the equivalent orifice diameter, g is the acceleration due to gravity, and *h* is the hydraulic head acting over the centreline of the orifice.

c) Parameter modified to variable

In a normal biofilter without RTC, the SZ depth is a design parameter often represented in models as a fixed value. However, with RTC, the SZ depth could be adjusted by discharging water from a bottom pipe at the base of a biofilter (this bottom pipe is normally included for the maintenance purpose; e.g., for Harvesting-Environmental RTC). In this case, h_{sz} is set as a variable in all the equations in the flow module.

d) Additional conditional judgment

In addition, to represent the trigger of control in the model, additional conditional judgment may need to be added (e.g., if-then statements). To achieve this, variables/RTC parameters (e.g., the instantaneous retention time of stormwater in biofilters) and the corresponding set-points/threshold to trigger control (e.g., minimum required retention time; e.g., for Harvesting RTC and Harvesting-Environmental RTC) should be determined.

It is noted that, after modification, the BioRTC model is able to represent all the operational behaviours in the laboratory experiments that described in Section 2.2.1. For example, the inflow equation modification (Eq. (12)) could simulate the inflow control Harvesting RTC, while outflow equation modification (Eq. (13)) and change SZ depth from a fixed parameter to a variable could reflect the operation of the bottom pipe. Moreover, the timing of open/closed the inflow/outflow pipe could be determined in the additional conditional judgement.

2.2.3 BioRTC model validation

Parameter calibration and sensitivity analyses were conducted on the BioRTC model using the dataset described in Section 2.2.1.

(1) Calibration of flow module

The parameters in flow module were calibrated with the data obtained from the RTC experiments described in Section 2.2.1. The ponding depths of each column was utilised to calibrate the parameter K_s (hydraulic conductivity), while the event outflow volumes were used to calibrate K_c (plant coefficient for evapotranspiration). Nash-Sutcliffe Efficiency was used to optimise the

parameter values and to evaluate the performance of the model (Nash and Sutcliffe, 1970), using the objective function that introduced in Shen et al. (2018b):

$$E_q = 0.5 \times (E_{depth} + E_{volume}) \qquad \text{Eq. (14)}$$

where E_q is the overall Nash-Sutcliffe Efficiency of the flow module (Nash and Sutcliffe, 1970); E_{depth} is the Nash-Sutcliffe Efficiency calculated using the observed and predicted ponding water depths (for K_s), and E_{volume} is the Nash-Sutcliffe Efficiency value calculated using the observed and predicted outflow volumes (for K_c).

(2) Calibration of microbial quality module

The microbial quality module was calibrated using the laboratory dataset presented in Section 2.2.1 as well. The Modified Monte-Carlo method that developed by Vezzaro et al. (2013) was adopted in calibration. 100,000 parameter sets were randomly generated. The parameters k_{att} (adsorption rate; 0.1 ~ 6 h^{-1}), μ_0 (standard die-off rate; 1 ~ 4 day^{-1}), and θ (temperature correction coefficient for die-off; 0.9 ~ 1.6) were uniformly distributed, while k_{det} (desorption rate; Log_{10} (k_{det}); -5 ~ 0.6 h^{-1}) was log-uniformly distributed. Each parameter set was used to predict the *E. coli* concentrations in the outflow of all of the columns (RTC biofilters + non-RTC biofilters) in the laboratory dataset. The method introduced by Vezzaro et al. (2013) was used to rank and select parameter sets. The criterion for parameter selection was to ensure the model prediction (the prediction bounds of all the selected parameters) intersects at least 70 % of the observations (observation uncertainty was 30 %, as per McCarthy et al. (2008)), and only limited increment in observation coverage could be achieved when further enlarging the number of parameter sets.

Nash-Sutcliffe Efficiency (E_c) was also adopted to evaluate the performance of water quality module in prediction. In addition, all the *E. coli* concentrations were log-transformed (as per NHMRC (2008)), thus helping to avoid biases caused by peak values (Criss and Winston, 2008).

2.3 Conceptualise new RTC strategies

To explore the potential of the new BioRTC model for designing new RTC strategies to meet the many competing objectives (Section 2), two new RTC strategies were conceptualised in this study, (1) Modified Harvesting RTC and (2) 1.5×SZ Harvesting-Environment RTC on biofilters, both of which are described below.

(1) Modified Harvesting RTC

Modified Harvesting RTC improves the original Harvesting RTC (Section 2.2.1) by delivering better environmental protection, while maintaining safe stormwater harvesting. The original Harvesting RTC only allows a volume of stormwater to enter the system which is less than or equal to the SZ volume (V_{sz}) to enter the biofilter (the rest is bypassed). However, there is also some extra space in the pore of unsaturated zone (USZ) that could be utilised to allow additional stormwater to enter into the biofilter (Figure 1). As such, the Modified Harvesting RTC utilises the spare space in the pores of USZ to allow additional stormwater to enter the biofilter, reducing the load that is bypassed to the environment. In addition, this strategy is expected to better balance the competing needs, as the additional water allows into biofilters may compensate evapotranspiration and enable more water for harvesting being collected. To fulfil this strategy, based on the facilities required for Harvesting RTC, only an extra moisture sensor to detect the saturation degree of USZ is needed. As such, this strategy still meets the low maintenance and low-cost equipment requirements for RTC (Shen et al., 2018a). To implement Modified Harvesting RTC, the first two control processes in Section 2.2.1 for Harvesting RTC remain unchanged, but the third process is modified to: when V_{in} reaches to V_{sz} plus the spare pore space of USZ, V_{pore} (i.e., when $V_{in} = V_{sz} + V_{pore}$), close the inflow valve and bypass the rest of stormwater into the environment. V_{pore} is calculated as $(S_{fc} - S_{usz}) \times V_{usz} \times n_{usz}$, where S_{fc} is the field capacity/maximum water holding capacity of filter media, S_{usz} is the saturation of USZ, V_{usz} is USZ volume, and n_{usz} is the porosity of USZ (Figure 1).

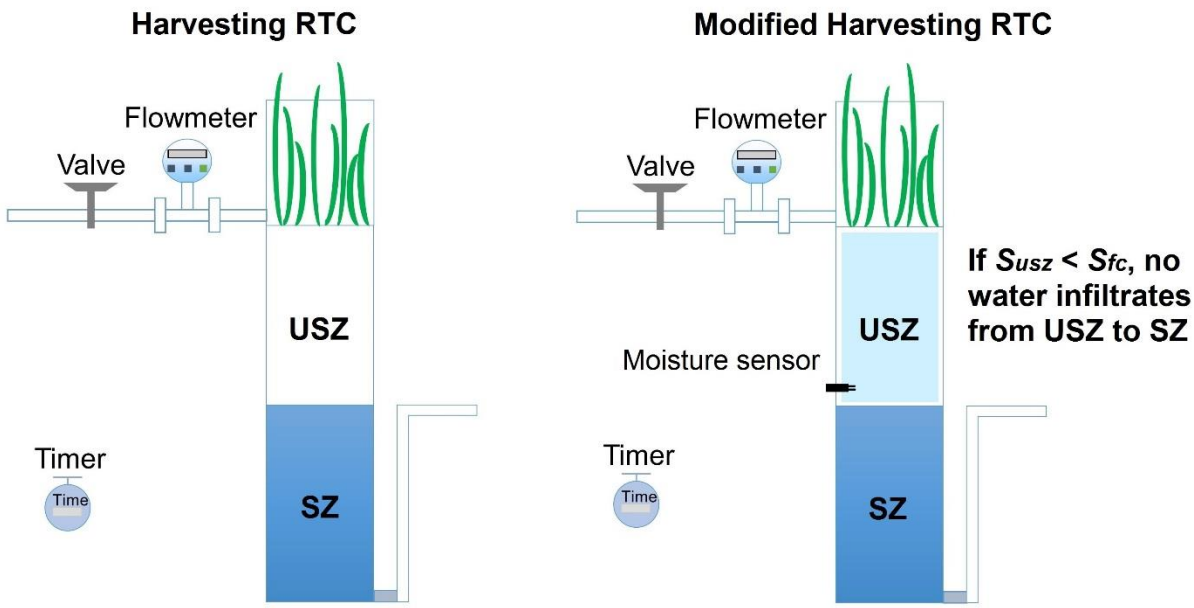


Figure 1 Schematic of Modified Harvesting RTC, with a comparison to Harvesting RTC. USZ: unsaturated zone; SZ: submerged zone.

(2) 1.5×SZ Harvesting-Environment RTC

The results of laboratory study introduced in Section 2.2.3 indicated that the water discharged in advance of harvesting was of high quality but only a limited amount of water was actually collected (Shen et al., 2018a). To increase the volume of water harvested, while maintaining its good quality and low bypass volumes, a potential solution is to enlarge the submerged zone (SZ) volume of the biofilters to retain more water. This 1.5×SZ Harvesting-Environment RTC employs the same rules of Harvesting-Environment RTC, but apply them to a biofilter with a 50% larger SZ pore volume (other design features remain unchanged, i.e. surface area, plant type, media type, and the depth of USZ). In this way, additional water is expected to be collected, and overflow could also be mitigated by the increased treatment capacity of biofilters. The implementation processes for 1.5×SZ Harvesting-Environment RTC are exactly the same to those for Harvesting-Environment RTC that were presented in Section 2.2.1.

2.4 Explore the potential of biofiltration RTC strategies using BioRTC

The two newly conceptualised RTC strategies (Section 2.3) were tested with the calibrated BioRTC model. For comparison's sake, the two previously

developed RTC strategies described in Section 2.2.1 were also tested. For all testing, a hypothetical biofilter system was assumed to be located at the outlet of the Hawthorn Main Drain West Catchment, Melbourne, Australia (a real catchment, where significant data on the hydraulics and *E. coli* levels exist; as per the Bureau of Meteorology, Australia and Melbourne Water Corporation). This catchment has 597 ha surface area, of which 45% is impervious.

The generation of flows and *E. coli* concentrations from this catchment was done using the Micro-Organism Prediction in Urban Stormwater model (McCarthy et al., 2011). For this, a two-year time series with 126 rainfall events (10/2012 - 09/2014) was used. MOPUS simulates the build-up and wash-off of microorganisms (McCarthy et al., 2011), and has two modules, a flow module and a water quality module, and both are explained in McCarthy et al. (2011). In brief, the flow module and the water quality module have five model parameters each, the values for all these parameters were adopted from Jovanovic et al. (2017). According to the simulation results, the *E. coli* concentrations in the inflow of the hypothetical biofilters ranged from 3.11×10^2 to 2.49×10^4 MPN/100 mL (mean concentration: 2.91×10^3 MPN/100 mL; standard deviation: 1.52×10^3 MPN/100 mL). Compared to the *E. coli* concentrations that spiked in the inflow during the laboratory experiments introduced in Section 2.2.1 (e.g., median concentrations: 3.09×10^4 MPN/100 mL and 6.46×10^4 MPN/100 mL; as per Shen et al. (2018a)), and the typical *E. coli* concentrations found around Melbourne (e.g., with a magnitude of 10^4 MPN/100 mL, as per McCarthy et al. (2008)), the median inflow concentration for the hypothetical biofilters were of one magnitude lower value.

The hypothetical biofilter which received this runoff was represented by the new BioRTC model and was assumed to have a surface area equal to 2% of the impervious catchment area for Melbourne's climate (as per FAWB (2009)). The other design features (i.e., media type, plant type, and the size of USZ) of the hypothetical biofilter system were exactly the same to those tested in the laboratory and described in Section 2.2.1. These were represented by the model parameters that were derived from the calibration process described in Section 2.2.3.

Four scenarios were generated, corresponding to the four RTC strategies (two previously developed and two newly conceptualised). It is noted that in each scenario, the minimum required retention time for retained water (T_{min}) for each strategy was re-evaluated by modelling, because the optimum T_{min} for a certain RTC strategy might be different when the strategy is applied to a different system. As such, thirteen T_{min} values were tested: 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 12 h, 18 h, 24 h, 30 h, 36 h, 42 h, and 48 h. The results under different T_{min} values were also compared with those when RTC was not implemented (non-RTC). It is noted that, same parameter sets for RTC under different T_{min} and non-RTC simulations were used, and all these parameter sets were obtained from the calibration that described in Section 2.2.3.

3. Results and discussion

3.1 Model validation using RTC data set

3.1.1 Flow module results

For the flow module, the calibrated K_s (hydraulic conductivity) ranged from 110 mm/h to 319 mm/h, somewhat matching those values suggested by Australian biofilter guidelines (e.g., 100 - 400 mm/h per FAWB (2009)). The calibrated K_c (plant coefficient for evapotranspiration) ranged from 0.6 to 1.0, and the difference was mainly due to the different plant growth status in different columns. E_q achieved in this study ranged from 0.72 to 0.91 (median: 0.84), which were comparable to those achieved in other studies for biofilter modelling (Shen et al., 2018b; Shen et al., 2018c) (the detailed results for the flow module are provided in Table C.1 in Supplementary Material C).

3.1.2 Microbial quality module results

3086 parameter sets were selected as after calibration, as they covered 70 % of the observations, and the cut-off point was where the marginal effect started to decrease (Figure C.1 in Supplementary Material C). When using this best-fit parameter set to predict the outcome of RTC (i.e., simulate the results of the laboratory experiments introduced in Section 2.2.3), the prediction fitted the observed *E. coli* concentrations in outflows well (Figure 2), as the vast majority of data points were scattered around the 1:1 line. With all these 3086 parameter sets, the prediction and observation fitted very well, as E_c ranged from 0.65 to

0.80. These E_c values were higher than those obtained in previous studies on the original model selected in Section 2.1 (on which BioRTC was built upon) (E_c = 0.46 ~ 0.55, according to Shen et al. (2018b) and Shen et al. (2018c)) or other stormwater *E. coli* modelling studies (e.g., E_c = 0.25 ~ 0.41 according to McCarthy et al. (2011)). The best-fit parameter values were all within the ranges that reported in literature (Table 3).

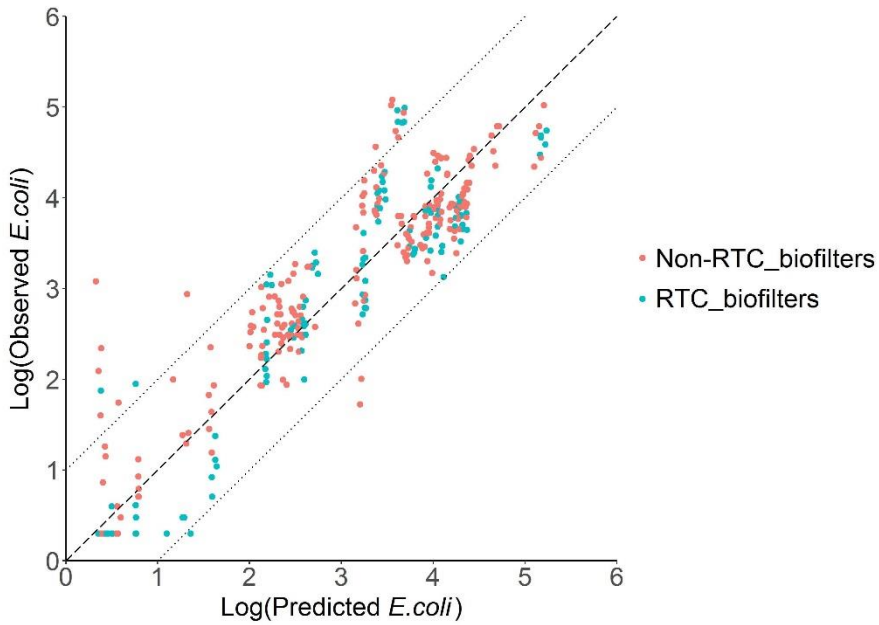


Figure 2 Comparison of observed and predicted *E. coli* concentrations in the outflow of all the columns and in all the events that were tested in laboratory experiments (with the best-fit parameter set). Dashed line indicates the 1:1 line between predicted and observed concentrations, while dotted lines indicate +/- one order of magnitude bars.

Table 3 The best-fit parameter values in the 3086 calibrated parameter sets and the corresponding E_c. Parameter values that reported in literature are provided for comparison.

	E_c	k_{att} (h^{-1})	Log (k_{det}) (h^{-1})	θ (-)	μ_0 (day^{-1})
The best-fit parameter set and corresponding E_c	0.80	0.20	-4.10	1.16	0.30
Parameter values reported in literature		0.20 ~ 5.86[a,b]	-4.22 ~ 0.31[a,b]	1.01 ~ 1.19[c]	0.06 ~ 1.23[d,e]

[a] Bradford et al. (2006); [b] Gargiulo et al. (2008); [c] Brauwere et al. (2014); [d] Chandrasena (2014); [f] Crane and Moore (1986)

In particular, with the best-fit parameter set, the median concentration of the harvested water collected from Harvesting RTC biofilters in laboratory experiments (introduced in Section 2.2.3) was slightly underestimated: 443 MPN/100 mL (predicted) vs. 740 MPN/100 mL (observed). The major reason for the underestimation was, in the laboratory experiments, preferential flow might occur due to cracks after dry days (Shen et al., 2018a). However, this difficult-to-model, and often random phenomenon was not captured in the model. As such, the model slightly underestimated the median concentration. When predicting the effects of Harvesting-Environment RTC implementation, the median concentration of harvested water was slightly overestimated: 3.5 MPN/100 mL (predicted) vs. < 1 MPN/100 mL (observed). Nevertheless, considering the small value of these concentrations, the difference (< 1 log) was still acceptable, well within the uncertainty in laboratory measurement methods (Chandrasena et al., 2012; Chandrasena et al., 2014b).

.

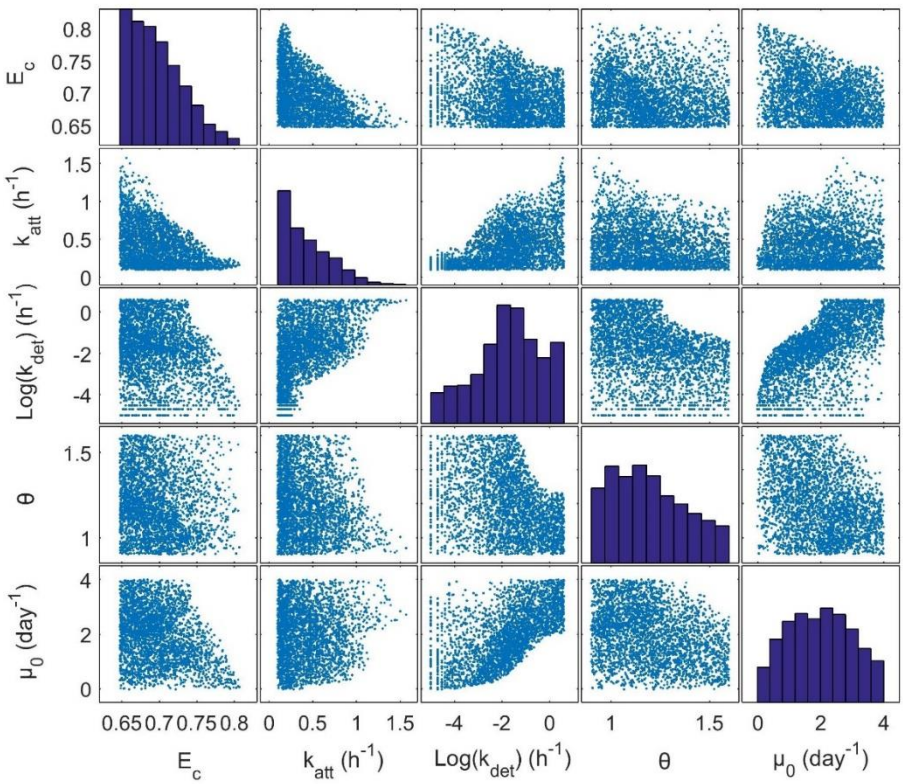


Figure 3 Matrix plot for parameter distributions of the 3086 parameter sets. The diagonal histograms represent the distributions of E_c and all the parameters, while the scatter plots between parameters reveal the parameter interactions.

The peak in the diagonal histograms of k_{att}, k_{det}, θ and μ_0 in the matrix plot (Figure 3) suggested that the model was sensitive to all the 3086 parameters in the water quality module (Dotto et al., 2012). These results indicated that adsorption, desorption, and die-off processes were crucial for microbial removal in stormwater biofilters incorporated with RTC, and temperature was an influential factor for die-off. All these findings agreed with those reported in the literature very well (Bradford et al., 2006; Chandrasena et al., 2014b; Stevik et al., 2004; Zhang et al., 2012).

More importantly, these findings demonstrated the hypothesis in previous studies on the same model that, better data quality could lead to better reflect the importance of parameters (Shen et al., 2018b; Shen et al., 2018c). In those previous studies, the same model was not sensitive to either temperature coefficient (θ) or standard die-off (μ_0), and it was hypothesised due to data paucity rather than model structure; while in the current study, when more

comprehensive data (e.g., more sampling events and more columns) were available, the importance of all the parameters were well reflected.

To explore the transferability of the BioRTC model, the prediction capability of the model for this dataset was also assessed using parameter sets that were obtained from calibration done in another independent study (Shen et al. (2018b)). In that study, the tested biofilters (without control) had similar design features (e.g., same plant type and filter media type) with those biofilters introduced in Section 2.2.1. The 3179 previously calibrated parameter sets were adopted to predict outflow concentrations of *E. coli*, the E_c values ranged from -0.07 to 0.70, and 64 % of them were above 0.40 (Figure 4). These promising E_c values indicated a high parameter transferability of BioRTC, as even the parameters calibrated for the biofilters without control could be adopted for the prediction of RTC biofilters.

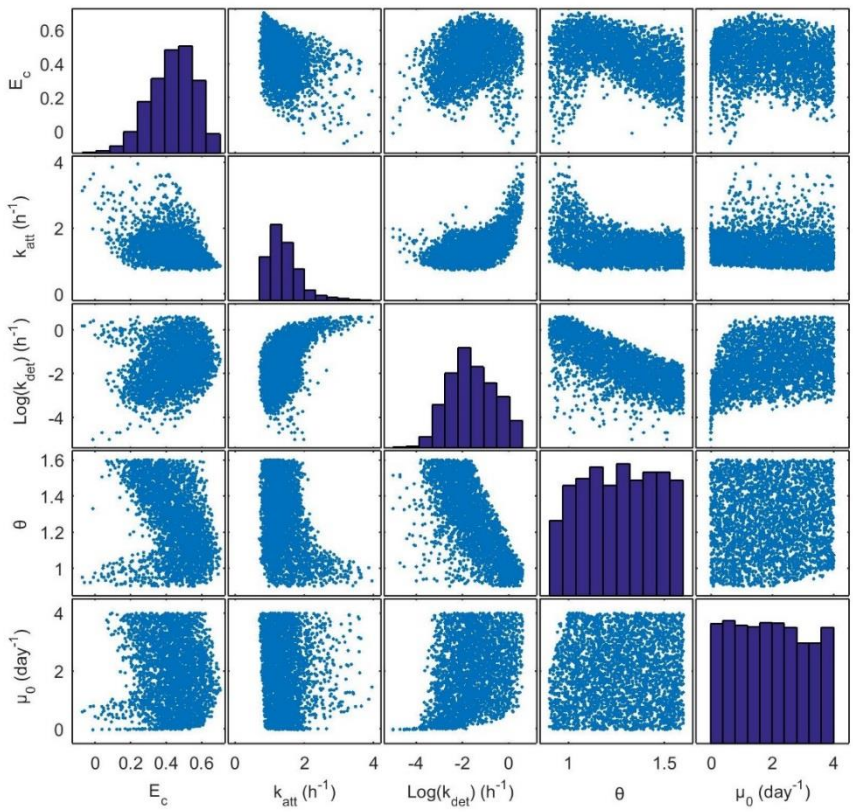


Figure 4 Matrix plot for parameter distributions of the 3179 parameter sets calibrated in Shen et al. (2018b). The diagonal histograms represent the distributions of E_c and all the parameters, while the scatter plots between parameters reveal the parameter interactions.

3.2 Scenario testing results with modelled representation of reality

3.2.1 Scenario 1: Harvesting RTC

Since each parameter generated a set of results, the 3086 parameter sets calibrated in the microbial quality module in Section 2.2 generated 3086 sets of results for each tested T_{min} in RTC systems and non-RTC simulation (Figure 5). With the increase of T_{min} value, the median *E. coli* concentrations and the 95th percentile of median *E. coli* concentrations in harvested water decreased (Figure 5-A), and the median log reductions of *E. coli* concentrations and the 5th percentile of median log reductions in harvested water increased (Figure 5-B). That was because, longer retention time could enhance the die-off of the trapped microbes in biofilters and provide better and more consistent water quality for stormwater harvesting. In addition, the proportions of load in the bypassed water increased and total load removal rates decreased with the increase of minimum required retention time (Figure 5-C), as more untreated stormwater was bypassed to the environment.

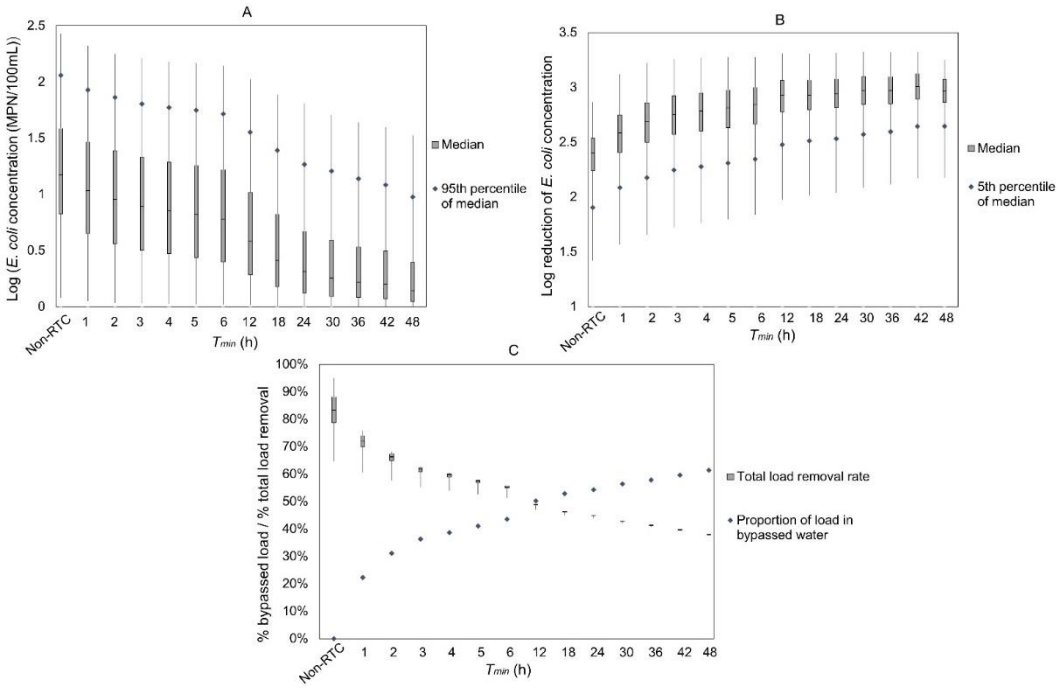


Figure 5 Boxplots for Harvesting RTC: the predicted median *E. coli* concentrations in harvested water (with 95th percentile marked) (A), median log reductions of *E. coli* concentrations in harvested water (with 5th percentile marked) (B), and proportions of load in bypassed water and total load removal rates (C), under different T_{min} values.

To select the optimum T_{min} for balancing the competing objectives in stormwater harvesting and reuse, the median *E. coli* concentration predicted with the best-fit parameter set in harvested water was plotted against the proportions of harvested water volume under different T_{min} values (Figure 6). The pattern of it was very similar to the plot that the best-fit prediction of total load removal rate against the proportions of harvested water volume under different T_{min} values, which was presented in the Supplementary Material (Figure D.1). A Pareto front was found, where it was impossible to optimize one objective (e.g., quality of harvested water) without making the other objective (e.g., the volume of harvested water) worse (Pareto, 1897). This Pareto front could help to decide the optimum T_{min} as a set-point, according to the specific case-study of interest. For example, if we aimed to harvest the water for dual reticulation with indoor and outdoor use or irrigation of commercial food crops (requirement: median concentration < 1 *E. coli*/100 mL, as per NHMRC (2009)), the optimum T_{min} was 24 h, as this set-point could help us collect the highest water volume under this water quality requirement (Figure 6); while if the harvested water is for municipal use with unrestricted access or irrigation of non-food crops (requirement: median concentration < 10 *E. coli*/100 mL, as per NHMRC (2009)), the optimum T_{min} was 2 h (Figure 6).

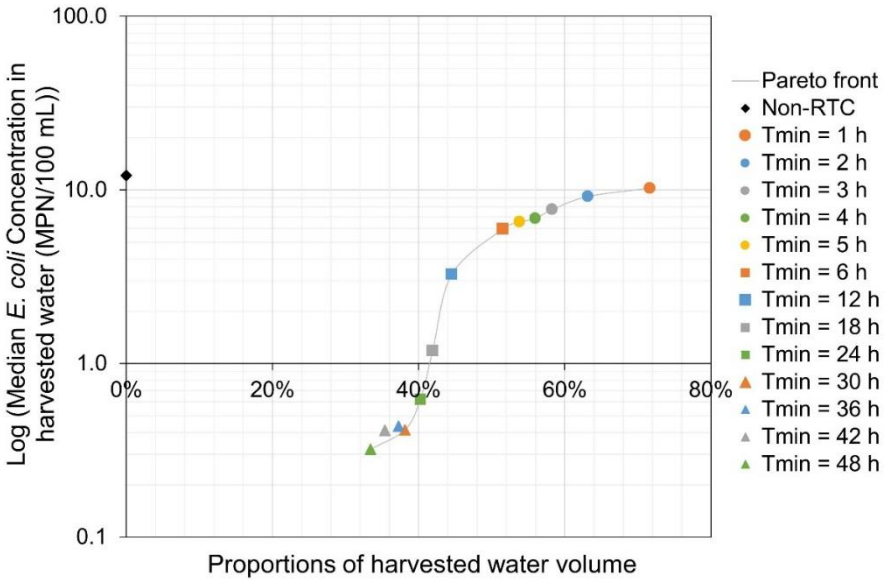


Figure 6 The best-fit prediction of median *E. coli* concentrations in harvested water against the proportions of harvested water volume, under different T_{min} values in Harvesting RTC.

When T_{min} was 2 h (i.e., to meet the requirement for municipal use with unrestricted access or irrigation of non-food crops), the volume of water collected for harvesting is 62.6 % of the stormwater runoff volume (Figure 6). According to Mitchell et al. (2008), this volume is sufficient to supply the non-seasonal uses plus seasonal uses in typical low-density residential areas in Melbourne (61.1 % volume required in total). Indeed, when T_{min} ranged from 1 h to 48 h, the proportions of harvested water ranged from 37.9 % to 70.5 %, which could significantly reduce potable water supply demand.

It is noted that, when T_{min} in current scenario was set as 48 h (which was same to the T_{min} value in the laboratory studies and simulations), a much better water quality in harvested water was achieved, compared to the result listed in Section 3.1.2 (median *E. coli* concentration: 0.32 MPN/100 mL vs. 443 MPN/100 mL). It was mainly due to the general lower *E. coli* concentrations in the inflow in the inputs of field scenarios. This result also indicated that, to meet a same target, the required T_{min} value could vary under different operational conditions (e.g., weather conditions and inflow characteristics), further demonstrating the necessity to testing T_{min} each time before RTC being implemented in a new system. To fulfil this need, the modelling framework in this study could be an efficient tool.

3.2.2 Scenario 2: Modified Harvesting RTC

For Modified Harvesting RTC, the change patterns of median concentrations, median log reductions, proportions of bypassed load, and total load removal rates under different T_{min} values were very similar to those for Harvesting RTC in Section 3.3.1, and the details are listed in Supplementary Material D (Figure D.2). Compared to the results for Harvesting RTC, the proportions of bypassed load slightly decreased (e.g., with the best-fit parameter set, under different the proportions of bypassed load, the proportions of bypassed load decreased by 0.4 ~ 1.5 %), as the spare pore space in USZ allowed more stormwater volume entering biofilters. Accordingly, the total load removal rates increased (0.5 ~ 0.9 %,with the best-fit parameter set), as the total load discharged to the environment mainly derived from the bypassed load. In addition, the volume of water collected for harvesting moderately increased, as the extra stormwater

that entered biofilters could compensate a portion of the water loss caused by evapotranspiration during dry days. For example, when T_{min} = 2 h, compared to those in Harvesting RTC, the proportion of load in bypassed water decreased by 0.8 %, and the proportion of harvested water volume increased by 0.4 %. Nevertheless, these harvested water was of diminished quality, as with the prediction using the best-fit parameter set, the median *E. coli* concentration increased and the median log reduction decreased. These might due to the additional stormwater entered biofilters introduced additional pollutants, which would cause that longer retention time is required for die-off enhancement to reach the same outflow quality level of that achieved in Harvesting RTC implementation. However, the diminishment in harvested water quality might be negligible, as when T_{min} = 2 h, with the best-fit parameter set, the decrement of median *E. coli* concentration was 0.2 MPN/100 mL, and the harvested water qualities could still respectively meet the requirements for municipal use with unrestricted access (NHMRC, 2009). Therefore, considering the higher water volume collected for harvesting, Modified Harvest RTC was recommended to be implemented with the inputs in this scenario, as compared to Harvesting RTC, the two objectives in stormwater harvesting and reuse (good quality and large amount of the water collected for harvesting) could be better balanced, and the environment could be better protected.

A Paleto front was also found in Figure D.4 for Modified Harvesting RTC. Although the pattern in Figure 6-right was also similar to that in Figure D.4, under a same T_{min} value, both the best-fit prediction of median *E. coli* concentration and proportion of harvested water volume in Figure 6-right were slightly higher than those in Figure D.4.

3.2.3 Scenario 3: Harvesting-Environment RTC

The change patterns of predicted median and 95th percentile *E. coli* concentrations in harvested water (Figure 7-A), mean and 5th percentile log reductions of *E. coli* concentrations in harvested water (Figure 7-B), all followed those achieved in Section 3.2.1 and Section 3.2.2. That was because, as longer retention time could enhance the die-off of *E. coli* in the water retained in biofilters. However, the total load removal rate remained consistent for non-

RTC and different T_{min} values in RTC (Figure 7-C). The reasons were proposed to be: (1) no stormwater was bypassed and all the inflow was treated by stormwater biofilters (Figure 7-C), and (2) under each T_{min} value, the volume of stormwater inflow that treated during the typical operation of biofilters (without control) were generally consistent and of high value, and the harvested water contained only negligible load (i.e., extremely low concentrations of *E. coli* were achieved, as per Figure 7-A). These results fitted previous findings in Shen et al. (2018a).

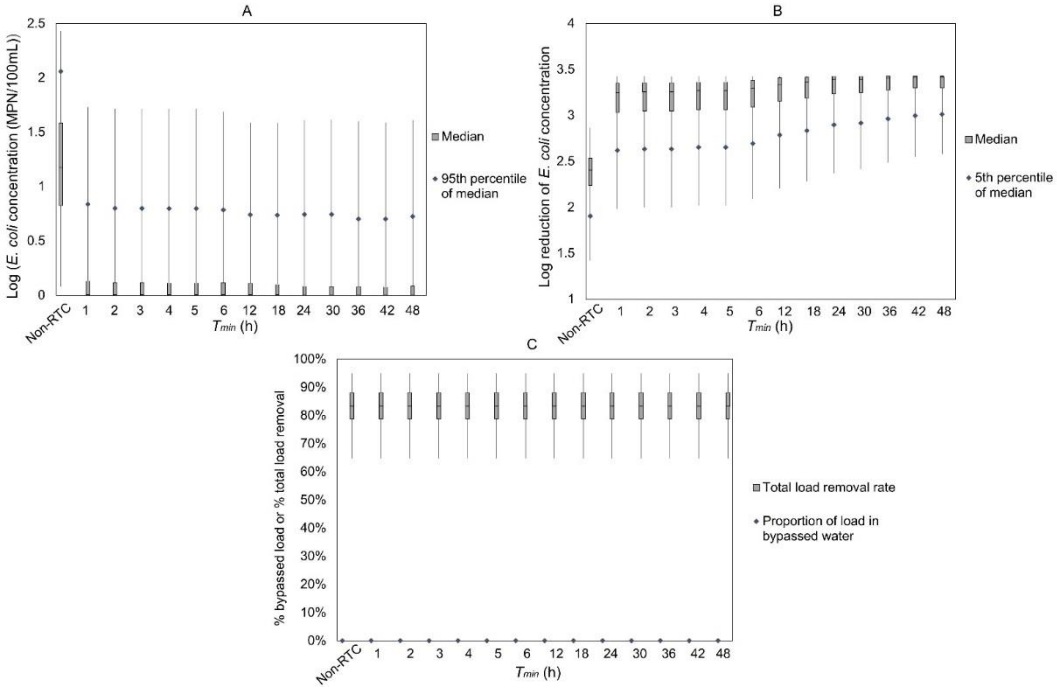


Figure 7 Boxplot for Harvesting-Environment RTC: the predicted median *E. coli* concentrations in harvested water (with 95th percentile marked) (A), median log reductions of *E. coli* concentrations in harvested water (with 5th percentile marked) (B), and proportions of load in bypassed water and total load removal rates (C), under different T_{min} values.

Even with 1-hour minimum retention time (T_{min} = 1 h), the best-fit prediction of median *E. coli* concentrations in harvested water was much lower than those from non-RTC biofilters' outflow. This concentration could meet the requirement for dual reticulation with indoor and outdoor use or irrigation of commercial food crops (median concentration < 1 *E. coli*/100 mL, as per NHMRC (2009)) (Figure 8-left). Compared to Harvesting RTC and Modified Harvesting RTC, less retention time is required for Harvesting-Environment to meet this requirement,

as the hydraulic gradient was lower when collecting the harvested water (i.e., discharge in advance, no ponding on the top of biofilters). With the increment of T_{min}, the water quality for harvesting was generally consistent (Figure 8-left), as additional retention time only provided minimum benefits for harvested water quality; while the proportion of harvested water volume decreased, as the biofilters operated as a non-RTC system more frequently.

Due to the extremely small differences in median *E. coli* concentrations under various T_{min}, there is no Pareto front and that no optimum could be found (Figure 8-left), and T_{min} = 1 h was suggested for this scenario. Similar patterns was found in the plot of total load removal rates against the proportions of harvested water volume (Supplementary Material D, Figure D.3).

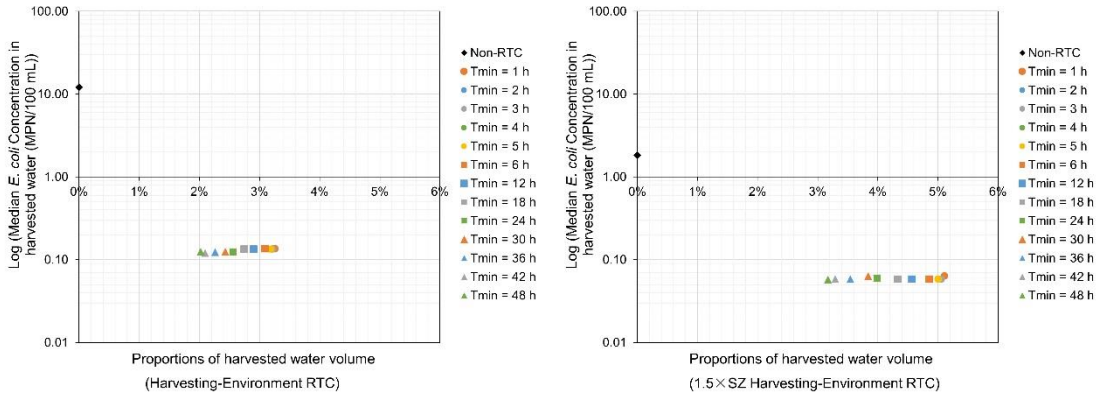


Figure 8 The best-fit prediction of median *E. coli* concentrations in harvested water against the proportions of harvested water volume, under different T_{min} values. Left: Harvesting-Environment RTC; right: 1.5×SZ Harvesting-Environment RTC.

It is noted that, even with 1 h T_{min}, only 3.3 % of stormwater was collected for harvesting (highest volume among all the T_{min}), due to the extensive inflow volume in individual event and extended dry length between rainfall events in the field inputs. Therefore, compared to those achieved in Harvesting RTC in Section 3.2.1**,** the benefits of Harvesting-Environment RTC were limited in this scenario, as to meet the requirement for dual reticulation with indoor and outdoor use or irrigation of commercial food crops (NHMRC, 2009), the maximum proportion of harvested water in Harvesting RTC implementation was

40.2 % (when T_{min} = 24 h), while that in Harvesting-Environment RTC implementation was just 3.3 % (when T_{min} = 1 h).

3.2.4 Scenario 4: 1.5×SZ Harvesting-Environment RTC

Compared to the results for Harvesting RTC on original field biofilters in Section 3.2.3, similar change patterns under different T_{min} values were found for all the key results, and these key results were listed in Supplementary Material D (Figure D.5). After the submerged zone volume increased to 1.5 times higher, under each T_{min} value, the volume of water collected for harvesting increased to around 1.5 times higher (e.g., when T_{min} = 1h, the volume of harvested water increased by 57 %), mainly due to the fact that larger SZ volume could retain more stormwater in the biofilters. More importantly, the harvested water was of even higher quality compared to the results obtained in Scenario 3 (biofilters with 1 SZ). The main reason was proposed to be that the higher SZ depth resulted in lower hydraulic gradient (could be well represented with Darcy's law in the model), therefore, the infiltration rates was lower, resulting in enhanced contact between *E. coli* and the filter media/plant roots. These results demonstrated the benefits of enlarging the submerged zone volume to achieve a better performance in the implementation of Harvesting-Environment RTC. Furthermore, the Pareto front analysis result was also same to that in Scenario 3 that there was no Pareto front and T_{min} = 1 h was suggested (Figure 8-right).

3.4 General discussion, limitations and future work

Establishing a deeper biofilter system is beneficial to stormwater harvesting and reuse, as a system with larger SZ depth could increase the water volume for harvesting by retain more water in SZ during dry days, and could improve the water quality as the hydraulic gradient is lower and accordingly the contact time is increased. It also could protect the environment better as a larger biofilter capacity could diminish the likelihood of overflow occurrence. These dynamics could be well reflected in the BioRTC model, and the modelling results have also demonstrated the benefits of enlarging SZ depth. Therefore, a biofilter will large SZ depth is always recommended in practice.

Utilising the USZ to allow more water enter biofilters until it is saturated could also be effective in reducing environmental pollution, as less untreated stormwater is bypassed. In addition, the additional water kept in USZ could compensate the evapotranspiration during dry days, enabling more harvestable water being collected in the next event. However, more studies need to be conducted on whether the quality of the water collected for harvesting would be an issue, as the inflow concentrations in the scenarios tested in this study were of low value, and a slight increment of *E. coli* concentrations in the outflow has been observed, although did not impact the water quality significantly. In the future, laboratory and field studies are suggested to test the impact under various level of inflow concentrations.

In addition, increasing the retention time is always a method to achieve low microbial concentration in the harvested water, and the water with sufficient retention time is recommend to be isolated with any newly entered stormwater with insufficient treatment time. However, longer retention time means the volume of harvested water might be decreased as a sacrifice. Moreover, extremely long retention might also negatively impact the removal of some other pollutants such as phosphorus (Shen et al., 2018a). Therefore, the required retention time should be evaluated case by case, according to the inflow concentrations and end use requirements.

4. Conclusion

The newly developed model in this study, BioRTC, has been proved to be effective in representing the outcome of RTC implementation. Therefore, this model could be employed to explore RTC strategies and scenarios. According to the simulation results using BioRTC, the two newly conceptualised RTC strategies, Modified Harvesting RTC and 1.5×SZ Harvesting-Environment RTC, were both effective to better protect the environment and better balance the competing needs (good water quality and large water volume for harvesting) in stormwater harvesting and reuse.

The benefits of a same RTC strategy and the corresponding optimum set-points might vary for different systems with different operational conditions (e.g., inflow

concentrations and dry lengths) and different treatment requirements (e.g., end uses). The new model, BioRTC, could be an efficient tool to select the most suitable RTC strategy and determine the optimum set-points.

Acknowledgement

The authors would like to thank Melbourne Water for generously sharing the data of the Hawthorn Main Drain West Catchment, Melbourne, Australia. The support of the Cooperative Research Centre for column establishment is gratefully acknowledged. The authors also wish to acknowledge the support of Dusan Jovanovic from Monash University in MOPUS simulation.

Reference

Allen R. G., Pereira L. S., Raes D., Smith M. Corp evapotranspiration - Guidelines for computing crop water requirements - FAO Irrigation and Drainage Paper No.56. FAO - Food and Agriculture Organization of the United Nations, Rome, Italy, 1998.

Bradford S. A., Simunek J., Walker S. L. Transport and straining of *E. coli* O157:H7 in saturated porous media. Water Resources Research 2006; 42: W12S12.

Bratieres K., Fletcher T. D., Deletic A., Zinger Y. Nutrient and sediment removal by stormwater biofilters: A large-scale design optimisation study. Water Research 2008; 42: 3930-3940.

Brauwere A. d., Ouattara N. K., Servais P. Modeling Fecal Indicator Bacteria Concentrations in Natural Surface Waters: A Review. Critical Reviews in Environmental Science and Technology 2014; 44: 2380-2453.

Chandrasena G. I. Faecal Microorganism Removal in Stormwater Biofilters. Department of Civil Engineering. Doctor of Philosophy. Monash University, 2014.

Chandrasena G. I., Deletic A., Ellerton J., McCarthy D. T. Evaluating *Escherichia coli* removal performance in stormwater biofilters: a laboratory-scale study. Water Science & Technology 2012; 66: 1132-1138.

Chandrasena G. I., Deletic A., McCarthy D. T. Evaluating *Escherichia coli* removal performance in stormwater biofilters: a preliminary modelling approach. Water Science & Technology 2013; 67: 2467-75.

Chandrasena G. I., Deletic A., McCarthy D. T. Survival of *Escherichia coli* in stormwater biofilters. Environmental Science and Pollution Research 2014a; 21: 391-401.

Chandrasena G. I., Deletic A., McCarthy D. T. Biofiltration for stormwater harvesting: Comparison of *Campylobacter spp.* and *Escherichia coli* removal under normal and challenging operational conditions. Journal of Hydrology 2016; 537: 248-259.

Chandrasena G. I., Pham T., Payne E. G., Deletic A., McCarthy D. T. *E. coli* removal in laboratory scale stormwater biofilters: Influence of vegetation and submerged zone. Journal of Hydrology 2014b; 519: 814-822.

Crane S. R., Moore J. A. Modeling Enteric Bacterial Die-off - A Review. Water, Air, and Soil Pollution 1986; 27: 411-439.

Criss R. E., Winston W. E. Do Nash values have value? Discussion and alternate proposals. Hydrological Processes 2008; 22: 2723-2725.

Czemiel Berndtsson J. Green roof performance towards management of runoff water quantity and quality: A review. Ecological Engineering 2010; 36: 351-360.

Davies C. M., Bavor H. J. The fate of stormwater associated bacteria in constructed wetland and water pollution control pond systems. Journal of Applied Microbiology 2000; 89: 349-360.

Dingman S. L. Physical Hydrology: Upper Saddle River, N.J. : Prentice Hall, 2002.

Dotto C. B. S., Mannina G., Kleidorfer M., Vezzaro L., Henrichs M., McCarthy D. T., et al. Comparison of different uncertainty techniques in urban stormwater quantity and quality modelling. Water Research 2012; 46: 2545-2558.

Duncan H. P. Urban Stormwater Quality: A Statistical Overview. Melbourne, Australia: Cooperative Research Centre for Catchment Hydrology, 1999.

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

Gargiulo G., Bradford S. A., Simunek J., Ustohal P., Vereecken H., Klumpp. E. Bacteria Transport and Deposition under Unsaturated Flow Conditions: The Role of Water Content and Bacteria Surface Hydrophobicity. Vadose Zone Journal 2008; 7: 406-419.

Hathaway J., Hhunt W., Graves A., Wright J. Field Evaluation of Bioretention Indicator Bacteria Sequestration in Wilmington, North Carolina. Journal of Environmental Engineering 2011; 137: 1103-1113.

Hathaway J., Hunt W. Indicator Bacteria Removal by Stormwater BMPs in Coastal North Carolina. World Environmental and Water Resources Congress: Challenges of Change, Providence, Rhode Island, United States, 2010, pp. 3218-3225.

Hatt B. E., Fletcher T. D., Deletic A. Hydrologic and pollutant removal performance of stormwater biofiltration systems at the field scale. Journal of Hydrology 2009; 365: 310-321.

Hsu N.-S., Huang C.-L., Wei C.-C. Multi-phase intelligent decision model for reservoir real-time flood control during typhoons. Journal of Hydrology 2015; 522: 11-34.

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

Jovanovic D., Hathaway J., Coleman R., Deletic A., McCarthy D. T. Conceptual modelling of E. coli in urban stormwater drains, creeks and rivers. Journal of Hydrology 2017; 555: 129-140.

Leon A. S., Kanashiro E. A., Valverde R., Sridhar V. Dynamic Framework for Intelligent Control of River Flooding: Case Study. Journal of Water Resources Planning and Management 2014; 140: 258-268.

Li Y. L., Deletic A., Alcazar L., Bratieres K., Fletcher T. D., McCarthy D. T. Removal of Clostridium perfringens, Escherichia coli and F-RNA coliphages by stormwater biofilters. Ecological Engineering 2012; 49: 137-145.

McCarthy D. T., Deletic A., Mitchell V. G., Diaper C. Development and testing of a model for Micro-Organism Prediction in Urban Stormwater (MOPUS). Journal of Hydrology 2011; 409: 236-247.

McCarthy D. T., Deletic A., Mitchell V. G., Fletchera T. D., Diaper C. Uncertainties in stormwater E. coli levels. Water Research 2008; 42: 1812-1824.

Meng Z., Chandrasena G., Henry R., Deletica A., Kolotelo P., McCarthy D. Stormwater constructed wetlands: A source or a sink of *Campylobacter* spp. Water Research 2018; 131: 218-227.

Mitchell V. G., D.T. McCarthy, Deletic A., Fletcher T. D. Urban stormwater harvesting e sensitivity of a storage behaviour model. Environmental Modelling & Software 2008; 23: 782-793.

Murphy H. M., Meng Z., Henry R., Deletic A., McCarthy D. T. Current Stormwater Harvesting Guidelines Are Inadequate for Mitigating Risk from *Campylobacter* During Nonpotable Reuse Activities. Environmental science & technology 2017; 51: 12498-12507.

NHMRC. Guidelines for Managing Risks in Recreational Water. Australian Government, National Health and Medical Research Council, Canberra, Australia, 2008.

NHMRC. Australian Guidelines for Water Recycling (Phase 2). Stormwater Harvesting and Reuse. Australian Government, Natural Resource Management Ministerial Council, Environment Protection and Heritage Council, National Health and Medical Research Council, Canberra, Australia, 2009.

Pareto V. The New Theories of Economics. Journal of Political Economy 1897; 5: 485-502.

Schilling W., Andersson B., Nyberg U., Aspegren H., Rauch W., Harremoës P. Real time control of wastewater systems. Journal of Hydraulic Research 1996; 34: 785-797.

Schütze M., Campisano A., Colas H., Schilling W., Vanrolleghem P. A. Real time control of urban wastewater systems—where do we stand today? Journal of Hydrology 2004; 299: 335-348.

Shen P., Deletic A., McCarthy D. T. Real time control of biofilters delivers stormwater suitable for harvesting and reuse Water Research (submitted) 2018a.

Shen P., Deletic A., Urich C., Chandrasena G. I., McCarthy D. T. Stormwater Biofilter Treatment Model for Faecal Microorganisms. Science of the Total Environment 2018b; 630: 992-1002.

Shen P., McCarthy D. T., Chandrasena G. I., Li Y., Deletic A. Validation and Uncertainty Analysis of a Stormwater Biofilter Treatment Model for Faecal Microorganisms. 2018c.

Stevik T. K., Aa K., Ausland G., Hanssen J. F. Retention and removal of pathogenic bacteria in wastewater percolating through porous media: a review. Water Research 2004; 38: 1355-1367.

Vezzaro L., Mikkelsen P. S., Deletic A., McCarthy D. Urban drainage models - simplifying uncertainty analysis for practitioners. Water Science & Technology 2013; 68: 2136-43.

Zhang L., Seagren E. A., Davis A. P., Karns J. S. The Capture and Destruction of *Escherichia coli* from Simulated Urban Runoff Using Conventional Bioretention Media and Iron Oxide-coated Sand. Water Environment Research 2010; 82: 701-714.

Zhang L., Seagren E. A., Davis A. P., Karns J. S. Long-term sustainability of *Escherichia coli* removal in conventional bioretention media. Journal of Environmental Engineering 2011; 137: 669-677.

Zhang L., Seagren E. A., Davis A. P., Karns J. S. Effects of temperature on bacterial transport and destruction in bioretention media: Field and laboratory evaluations. Water Environment Research 2012; 84: 485-496.

Zinger Y., Deletic A., Fletcher T. D., Breen P., Wong T. A Dual-mode Biofilter System: Case study in Kfar Sava, Israel. 12th International Conference on Urban Drainage, Porto Alegre, Brazil, 2011.

Supplementary material A: Full details of model description and equations

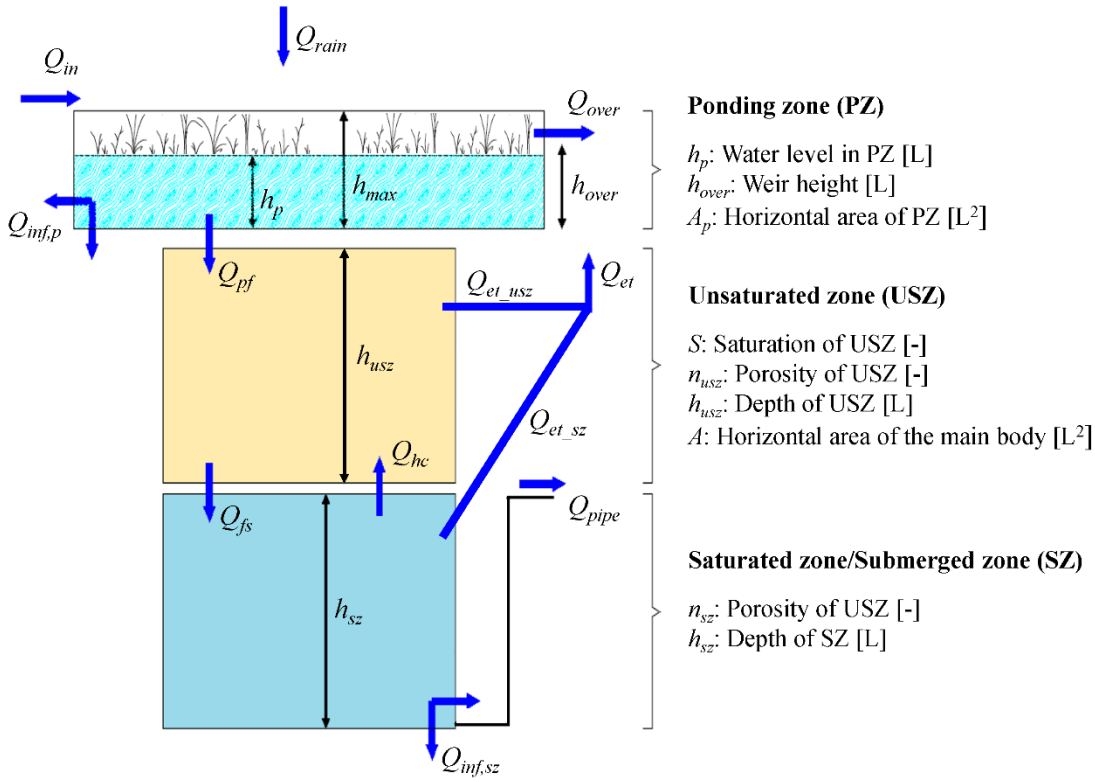


Figure A.1 Schematic representation of a typical stormwater biofilter and its flow scheme, and the key variables in each part. Stormwater inflow (Q_{in}), rainfall precipitation (Q_{rain}), overflow (Q_{over}), water flow from PZ to USZ (Q_{pf}), flow from the USZ to SZ (Q_{fs}), evapotranspiration (Q_{et}; divided to the evapotranspiration from USZ (Q_{et_usz}) and from SZ (Q_{et_sz})), capillary rise (Q_{hc}), infiltration into the surrounding soil ($Q_{inf,p}$ and $Q_{inf,sz}$) and outflow (Q_{pipe}).

Table A.1 Equations for the flow module.

Flow module equation	Eq. No.
General form of equations	
Flow = min (physically possible; available upstream; available downstream)	
Ponding zone (PZ)	
Infiltration from PZ to unsaturated zone	
$Q_{pf} = \min\left(K_s A \frac{h_p + h_{usz}}{h_{usz}}, \frac{h_p A_p}{dt} + Q_{in} + Q_{rain}, \frac{1}{dt}(1-S) n_{usz} h_{usz} A\right)$	(A.1)
Infiltration from PZ to the surrounding soil	
$Q_{inf,p} = \begin{cases} \min\left(K_f\left[(A_p - A) + C_s h_p P_p\right], \frac{h_p A_p}{dt}\right), \text{if unlined} \\ 0, \text{if lined} \end{cases}$	(A.2)
Overflow through weirs	
$Q_{over} = \begin{cases} \min\left(C_Q B \sqrt{2g(h_p - h_{over})^3}, \frac{A_p(h_p - h_{over})}{dt}\right), h_p > h_{over} \\ 0, h_p \le h_{over} \end{cases}$	(A.3)
Water mass balance in PZ	
$\frac{d(h_p A_p)}{dt} = Q_{in} + Q_{rain} - Q_{pf} - Q_{over} - Q_{inf,p}$	(A.4)
Unsaturated zone and saturated zone (USZ and SZ)	
Entire Saturation in USZ plus SZ	
$S_{entire} = \frac{S \times n_{usz} h_{usz} + n_{sz} h_{sz}}{n_{usz} h_{usz} + n_{sz} h_{sz}}$	(A.5)
Total Evapotranspiration from USZ and SZ	
$Q_{et} = \begin{cases} 0, S_{entire} \le S_w \\ A \times K_c \times ET_0 \frac{S_{entire} - S_w}{S_s - S_w}, S_w < S_{entire} \le S_s \\ A \times K_c \times ET_0, S_s < S_{entire} \le 1 \end{cases}$	(A.6)
Unsaturated zone (USZ)	
Evapotranspiration from USZ	
$Q_{et\_usz} = Q_{et} \times \frac{S \times n_{usz} h_{usz}}{S \times n_{usz} h_{usz} + n_{sz} h_{sz}}$	(A.7)
Flow due to capillary rise	
$Q_{hc} = A C_r (S - S_s)(S_{fc} - S), C_r = \frac{4 \times K_c \times ET_0}{2.5(S_{fc} - S_s)^2}$	(A.8)
when $S_s \le S \le S_{fc}$, otherwise $Q_{hc} = 0$	
Infiltration from USZ to SZ	
$Q_{fs} = \begin{cases} \min\left(A \times K_s \frac{h_p + h_{usz}}{h_{usz}} S^{\gamma}, \frac{(S - S_{fc}) A \times n_{usz} h_{usz}}{dt} + Q_{pf} + Q_{hc}\right), S \ge S_{fc} \\ 0, S < S_{fc} \end{cases}$	(A.9)
Water mass balance in USZ	
$\frac{d(S \times n_{usz} h_{usz} A)}{dt} = Q_{pf} + Q_{hc} - Q_{fs} - Q_{et\_usz}$	(A.10)
Saturated zone (SZ)	
Evapotranspiration from SZ	
$Q_{et\_sz} = Q_{et} \times \frac{n_{sz} h_{sz}}{S \times n_{usz} h_{usz} + n_{sz} h_{sz}} = Q_{et} - Q_{et\_usz}$	(A.11)
Infiltration from SZ to the surrounding soil	
$Q_{inf,sz} = \begin{cases} \min\left(K_f (A + C_s P_{sz} h_{sz}), \frac{n_{sz} h_{sz} A}{dt}\right), \text{if unlined} \\ 0, \text{if lined} \end{cases}$	(A.12)
Flow through drainage pipe	
$Q_{pipe} = \begin{cases} \min\left(A \times K_s \frac{h_p + h_{usz}}{h_{usz} + h_{sz}}, \frac{(h_{sz} - h_{pipe}) n_{sz} A}{dt} + Q_{fs} - Q_{hc} - Q_{et\_sz} - Q_{inf,sz}\right), h_{sz} > h_{pipe} \\ 0, h_{sz} \le h_{pipe} \end{cases}$	(A.13)

Table A.2 Equations for the microbial quality module.

Microbial quality module equation	Eq. No.
Ponding zone (PZ)	
Microbial mass balance in PZ	
$\frac{d(c_p h_p A_p)}{dt} = c_{in} Q_{in} - c_p(Q_{pf} + Q_{over} + Q_{inf,p}) - \mu c_p h_p A_p$	(A.14)
Unsaturated zone (USZ)	
Microbial mass balance in the water phase	
$\frac{\partial(Sn_{usz}c_{usz})}{\partial t} + (Sn_{usz}k_{att}c_{usz} - \rho k_{det}M_1) = \frac{\partial}{\partial z}\left(Sn_{usz}D_1\frac{\partial c_{usz}}{\partial z}\right) - \frac{\partial(q_1 c_{usz})}{\partial z} - Sn_{usz}\mu c_{usz}$	(A.15)
Adsorption, desorption and die-off of adsorbed microbes in the soil phase	
$\frac{\partial M_1}{\partial t} = \frac{n_{usz}S}{\rho}k_{att}c_{usz} - k_{det}M_1 - \mu M_1$	(A.16)
Dispersion coefficient in USZ	
$D_1 = \lambda\frac{q_1}{n_{usz}S}$	(A.17)
Average unit flow in USZ	
$q_1 = \frac{\alpha_1(Q_{pf} - Q_{et}) + \beta_1(Q_{fs} - Q_{hc})}{A}$	(A.18)
where $\alpha_1 + \beta_1 = 1$, and $\alpha_1 = 1$ at upper boundary, $\beta_1 = 1$ at lower boundary	
Saturated zone (SZ)	
Microbial mass balance in the water phase	
$\frac{\partial(n_{sz}c_{sz})}{\partial t} + (n_{sz}k_{att}c_{sz} - \rho k_{det}M_2) = \frac{\partial}{\partial z}\left(n_{sz}D_2\frac{\partial c_{sz}}{\partial z}\right) - \frac{\partial(q_2 c_{sz})}{\partial z} - n_{sz}\mu c_{sz}$	(A.19)
Adsorption, desorption and die-off of adsorbed microbes in the soil phase	
$\frac{\partial M_2}{\partial t} = \frac{n_{sz}}{\rho}k_{att}c_{sz} - k_{det}M_2 - \mu M_2$	(A.20)
Dispersion coefficient in SZ	
$D_2 = \lambda\frac{q_2}{n_{sz}}$	(A.21)
Average unit flow in SZ	
$q_2 = \frac{\alpha_2(Q_{fs} - Q_{hc}) + \beta_2(Q_{pipe} + Q_{inf,sz})}{A}$	(A.22)
where $\alpha_2 + \beta_2 = 1$, and $\alpha_2 = 1$ at upper boundary, $\beta_2 = 1$ at lower boundary	
Die-off rate in each part	
$\mu = \mu_0\theta^{T-20℃}$	(A.23)

Table A.3 Parameters in the model. Input parameters: parameters based on design and measurement. Calibration parameters: parameters calibrated in this study.

Flow module parameters		**Microbial quality module parameters**	
Input parameters			
B	Length of overflow weir [L]	ρ	Bulk soil density [M L^{-3}]
P_p	Unlined perimeter [L]	λ	Dispersivity [L]
C_Q	Weir overflow coefficient [-]		
C_s	Side infiltration coefficient [-]		
K_f	Hydraulic conductivity of the surrounding material [L T^{-1}]		
S_w	Wilting point [-]: washed sand, 0.05; loamy sand, 0.07		
S_s	Saturation as the threshold for plants to reach potential evapotranspiration [-]: without SZ, 0.22; with SZ, 0.37		
S_{fc}	USZ saturation at field capacity [-]: without SZ, 0.37; with SZ, 0.61		
γ	Relative hydraulic conductivity coefficient dependent on soil type [-]: washed sand, 11.1; loamy sand, 11.76		
Calibration parameters			
K_s	Hydraulic conductivity of the filter media [L T^{-1}]	k_{att}	Adsorption rate [T^{-1}]
K_c	Plant coefficient for evapotranspiration [-]	k_{det}	Desorption rate [T^{-1}]
		μ_0	Standard die-off rate at given reference conditions (e.g., standard temperature) [T^{-1}]
		θ	Temperature correction coefficient for die-off [-]

Supplementary material B: Schematics of Harvesting RTC rules, Harvesting-Environment RTC rules, and a biofilter column in laboratory experiments

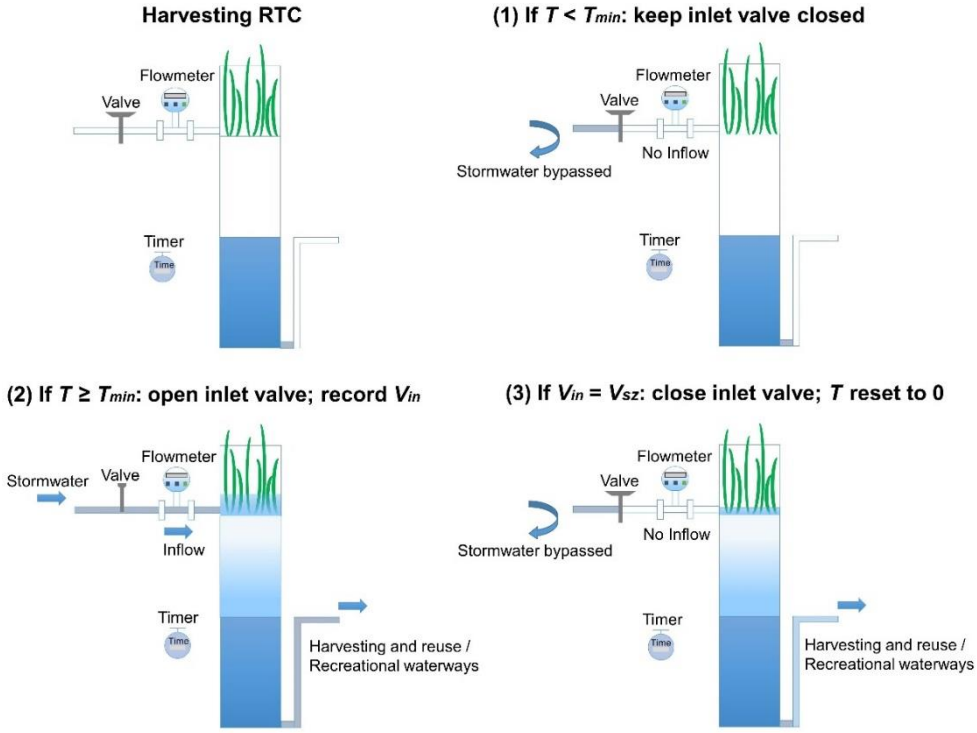


Figure B.1 Schematic of Harvesting RTC rules. T: stormwater retention time; T_{min}: minimum required retention time; V_{in}: stormwater volume that has entered biofilters; V_{sz}: submerged zone's pore volume. Top left shows the required facilities to fulfil this strategy (i.e., a flow meter, a valve, and a timer). When stormwater runoff comes: if $T < T_{min}$, keep the valve closed and bypass all the stormwater (top right); if $T \geq T_{min}$, open the valve and start to record V_{in} (bottom left). When $V_{in} = V_{sz}$ (i.e., submerged zone's pore volume will be fully replaced with newly entered stormwater), close the valve and bypass the rest of stormwater (bottom right).

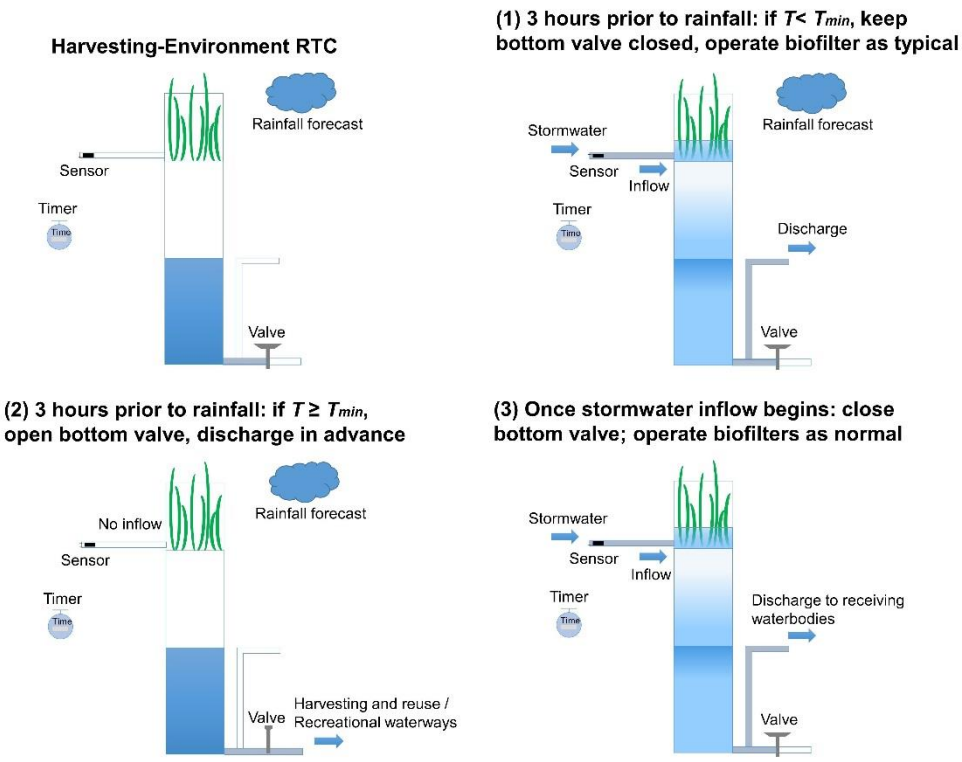


Figure B.2 Schematic of Harvesting-Environment RTC rules. T: stormwater retention time; T_{min}: minimum required retention time. Top left shows the required facilities and information to fulfil this strategy (i.e., a sensor, a valve, a timer, and rainfall forecast). When rainfall forecast informs that the next wet event will occur in three hours: if $T < T_{min}$, keep the valve closed and operate biofilters as typical, and the treated stormwater is discharged through the raised outflow pipe (top right); if $T \geq T_{min}$, open the valve and collected the harvestable water through free draining (bottom left). Once stormwater inflow begins, biofilters operate as typical again (bottom right).

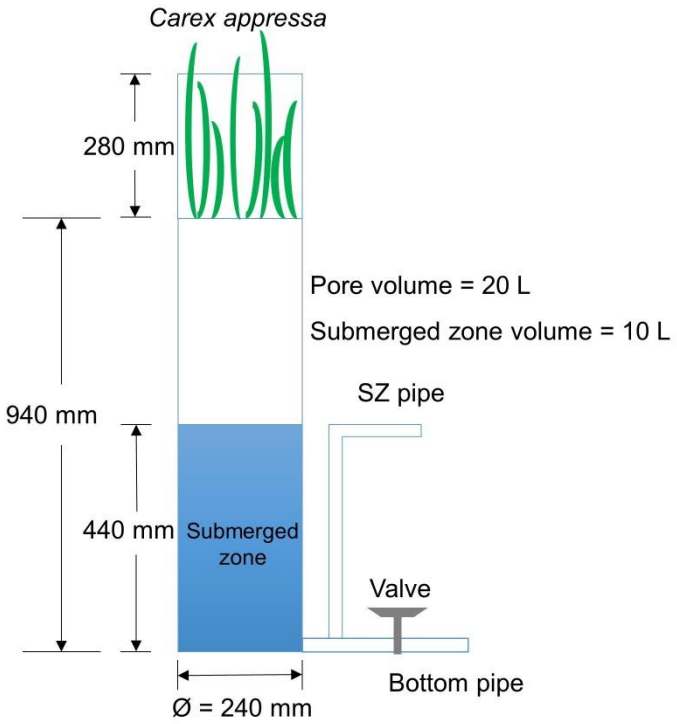


Figure B.3 Schematic of a biofilter column in laboratory experiments.

Supplementary material C: Results for the calibration of flow module

Table C.1 Calibrated K_s and K_c, and corresponding E_{depth}, E_{volume} and E_q for each RTC and non-RTC biofilters.

Biofilter type		Replicate1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
RTC biofilters	K_s (mm/h)	319	166	221	172	251
	E_{depth}	0.58	0.80	0.56	0.84	0.69
	K_c (-)	0.6	0.6	0.6	0.6	0.8
	E_{volume}	0.89	0.75	0.87	0.77	0.89
	E_q	0.73	0.77	0.71	0.81	0.79
Non-RTC biofilters	K_s (mm/h)	150	188	122	110	132
	E_{depth}	0.75	0.80	0.84	0.81	0.81
	K_c (-)	0.7	1.0	0.8	0.8	0.8
	E_{volume}	0.98	0.97	0.98	0.97	0.97
	E_q	0.86	0.88	0.91	0.89	0.89

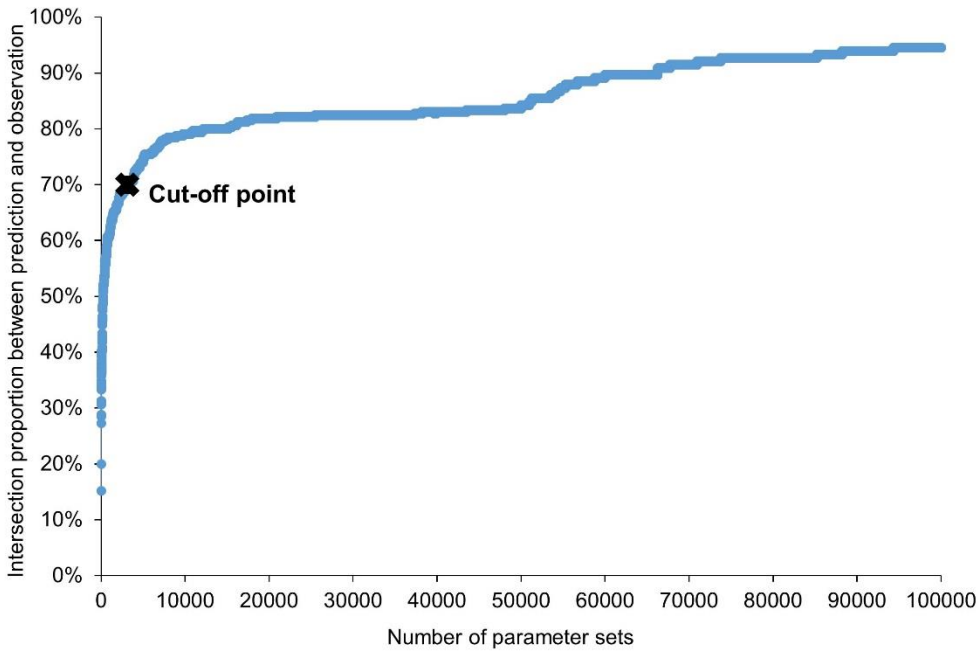


Figure C.1 Cut-off points for parameter selection when re-calibrating parameters for the microbial quality module in current study.

Supplementary material D: Supplementary results in scenario testing

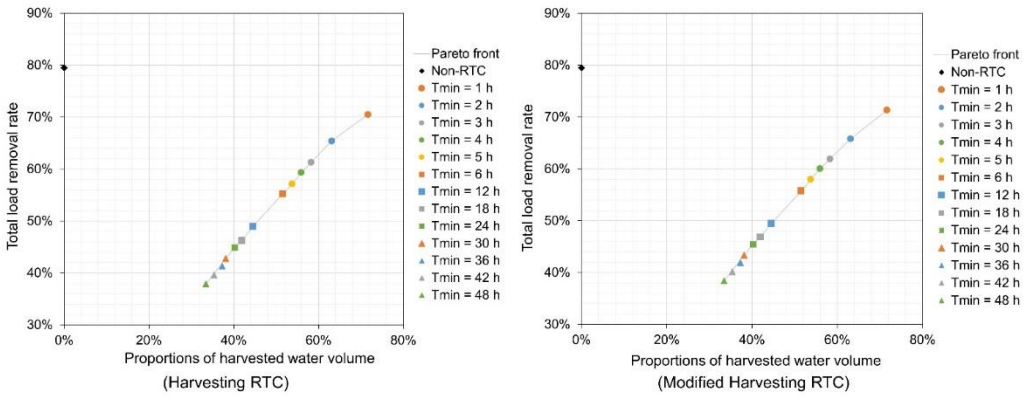


Figure D.1 The best-fit prediction of total load removal rates against the proportions of harvested water volume, under different T_{min} values. Left: Harvesting RTC; right: Modified Harvesting RTC.

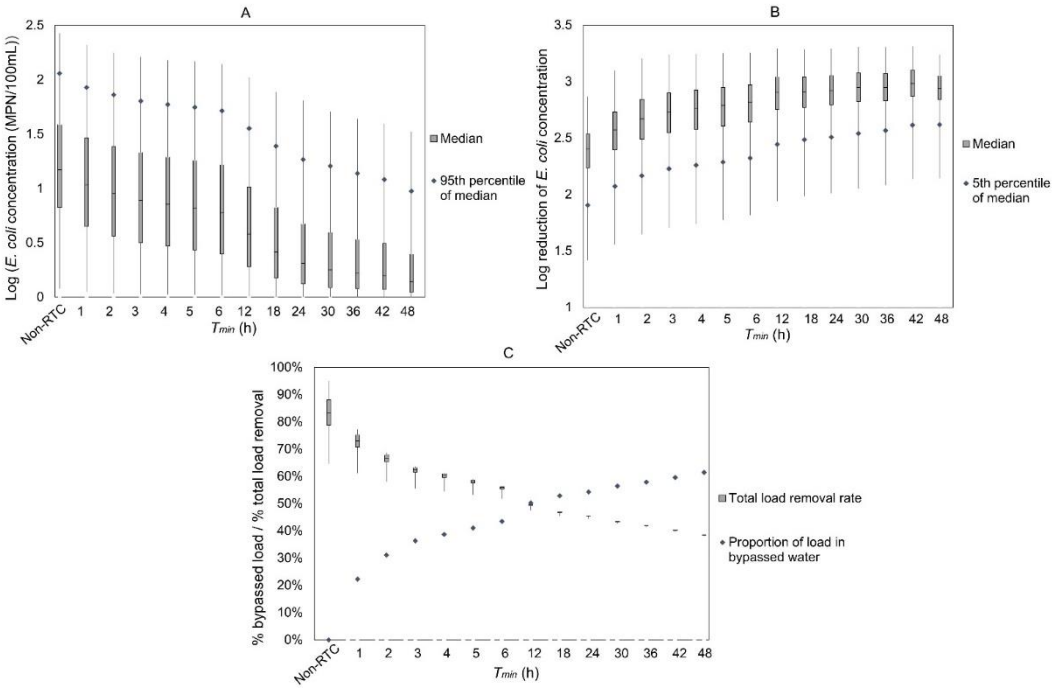


Figure D.2 Boxplots for Modified Harvesting RTC: the predicted median *E. coli* concentrations in harvested water (with 95th percentile marked) (A), median log reductions of *E. coli* concentrations in harvested water (with 5th percentile marked) (B), proportions of load in bypassed water (C), and total load removal rates (D), under different T_{min} values.

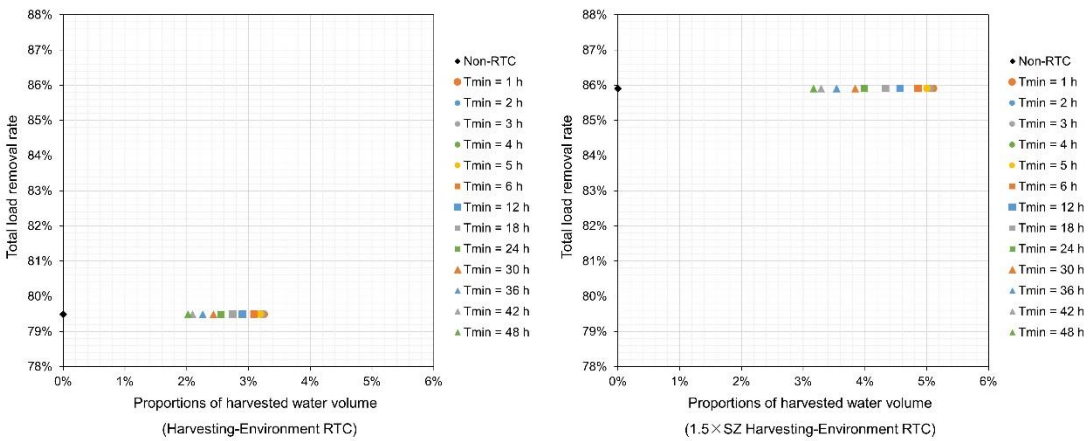


Figure D.3 The best-fit prediction of total load removal rates against the proportions of harvested water volume, under different T_{min} values. Left: Harvesting-Environment RTC; right: 1.5×SZ Harvesting-Environment RTC.

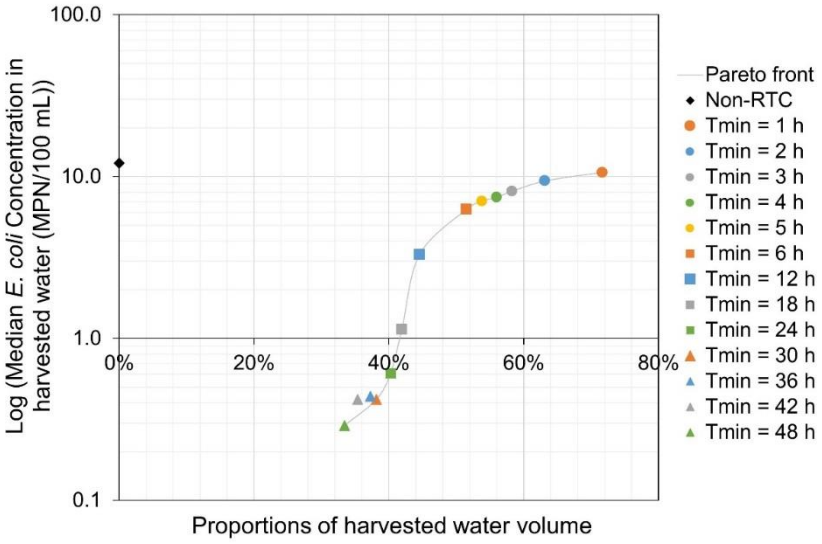


Figure D.4 The best-fit prediction of median *E. coli* concentrations in harvested water against the proportions of harvested water volume, under different T_{min} values in Modified Harvesting RTC.

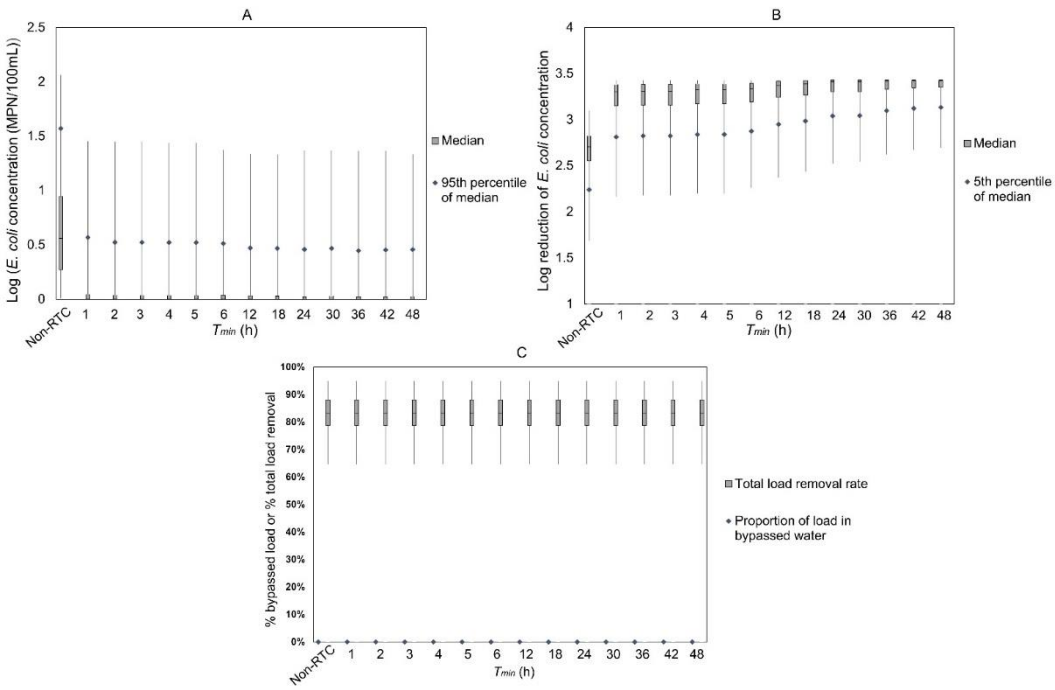


Figure D.5 Boxplots for 1.5×SZ Harvesting-Environment RTC: the predicted median *E. coli* concentrations in harvested water (with 95th percentile marked) (A), median log reductions of *E. coli* concentrations in harvested water (with 5th percentile marked) (B), and proportions of load in bypassed water and total load removal rates (C), under different T_{min} values.

6.3 Discussion and conclusions

The good fit between the RTC outcome that observed during laboratory experiments and the prediction by a new model, BioRTC that developed by modifying the model introduced in Chapter 3 demonstrated that, BioRTC is effective in RTC assessment. More importantly, when the parameters calibrated for non-RTC biofilters were employed for RTC prediction, promising results could still be achieved, indicated the high parameter transferability of model in RTC simulation. This finding further demonstrated the feasibility of using BioRTC to explore RTC strategies and scenarios, especially in the case that when RTC is proposed to be implemented in a brand new system, where no data are available for parameter calibration.

As such, the new model BioRTC could be utilised as the fundamental part of the framework for RTC in stormwater biofilters. With this modelling tool, additional strategies and scenarios could be tested and evaluated, to better balance the competing needs existed in RTC implementation (e.g., water volume and water quality for harvesting).

The two RTC strategies developed in Chapter 5, and the additional conceptual strategies developed in the study presented in this chapter, were simulated with a modelled representation of reality. The modelling results suggested that the newly developed RTC were capable of balancing the competing needs in stormwater harvesting better. In addition, different set-points of a same strategy could result in different outputs, indicating the necessity of testing RTC strategies and corresponding set-points case by case, to determine the optimum RTC and operational scheme. To fulfil this goal, again, the modelling tool BioRTC would be an economical and efficient option.

Based on all the findings above, it is concluded that, the framework of RTC for stormwater biofilters by developing and utilising modelling tool has successfully been developed and proved to be effective.

However, there are still some studies need to be furthered. For example, although the developed strategies were tested using a modelled representation of reality, the benefits of RTC in the field would be better revealed if field tests could be conducted in practical. This work is expected to be carried out in the future. Considering that (1) BioRTC is capable of predicting the performance of laboratory-scale RTC biofilters, (2) the model developed in Chapter 3 has been demonstrated to be transferable from laboratory-scale to field-scale for non-RTC biofilters, and it has a same structure with BioRTC, and (3) there are plenty of similarities between the operation of RTC and non-RTC biofilters, it is expected that the performance of RTC systems in the field could be also well reflected by BioRTC. In addition, more RTC strategies and scenarios will be explored and tested with the BioRTC modelling tool as well.

Chapter 7.

Conclusions and future work

7.1 Introduction

This chapter summarises the key findings for the study of RTC of stormwater biofilters that were presented in the previous chapters. Practical implications and suggestions, and strengths and limitations of this study, are also highlighted. Lastly, recommendations for future work are provided.

7.2 Key findings

This research aimed to develop a framework for real time control to achieve operational optimisation of stormwater biofilters. To achieve this aim, modelling and experiments were integrated, and the key findings of this study are presented as below.

(1) Development and testing of stormwater biofilter treatment model for faecal microorganisms

The model developed in Chapter 3 includes three major processes (i.e., adsorption, desorption, and die-off) and one operational factor (i.e., temperature) that govern the microbial removal in biofilters. Compared to the previous models in this field, the model developed in this study is a rare one that could provide a long-term simulation for biofilters with different designs features and under a wide range of operational conditions.

The model was tested with 44-week-long laboratory experiments on five different biofilter configurations (two media types and four plant types), and the prediction showed good agreement with the observation. Promising Nash-Sutcliffe Efficiency (E_c) values were achieved when comparing the prediction with observation, ranging from 0.46 to 0.68. In addition, all the calibrated model parameter values were within the range of those reported in literature.

The sensitivity analyses results indicated that adsorption and desorption processes were dominant in microbial removal. However, due to insufficient data available for calibration, correlations between different parameters were found. The validation results further demonstrated that more data were required

to adequately test the model. Therefore, it is suggested to calibrate and validate the model with more data sets.

(2) Model validation, parameter transferability analysis, and uncertainty analysis

The stormwater biofilter treatment model for faecal microorganisms that developed in Chapter 3 was successfully validated with the independently collected data sets from lab-scale and field-scale biofilters with various designs and operational schemes (Nash Sutcliffe Efficiency E_c: 0.50 ~ 0.60), demonstrating the broad application of this model.

The sensitivity analysis results reinforced the significance of adsorption and desorption to microbial removal in stormwater biofilters. Furthermore, the importance of temperature to die-off, which was not fully represented in the previous study, was also revealed. This finding indicated that better data quality could better reflect the importance of parameters.

Most importantly, a high parameter transferability of this model was found, as the prediction agreed with observation well, even if the parameters from another system with similar plant type and media type were adopted. The results of uncertainty analysis further demonstrated the low uncertainty of model prediction when parameter values from another system were adopted.

Particularly, the model could be applied to large-scale field systems after being calibrated with only simple laboratory tests, as a good agreement between prediction and observation with low prediction uncertainty was achieved (E_c = 0.55, intersection percentage between the prediction uncertainty bands and observation with uncertainty intervals = 83 %), when the parameters calibrated from a laboratory study were employed to predict the performance of a field system. Considering that stormwater biofilters are often large systems that cannot be easily validated, the model could be widely utilised as a valuable tool for system evaluation.

(3) Development and laboratory testing of real time control strategies for stormwater biofilters

The two developed RTC strategies, Harvesting RTC and Harvesting-Environment RTC, were both effective in reducing the risks posed by faecal microbes during stormwater harvesting and reuse in the laboratory column studies: after implementing Harvesting RTC, the outflow from RTC biofilters (i.e., biofilters implemented with RTC) for harvesting could meet the secondary contact for recreational water use (median *E. coli* concentration < 1000 MPN/100 mL); after implementing Harvesting-Environment RTC, the outflow from RTC biofilters for harvesting could fulfil the requirements for dual reticulation with indoor and outdoor use or irrigation of commercial food crops (median *E. coli* concentration < 1 MPN/100 mL; median turbidity < 25). However, without RTC, none of these requirements could be met.

Harvesting RTC collected much more water for harvesting than Harvesting-Environment RTC (proportions of water collected for harvesting: 28.4 % vs. 5.40 %). However, 60 % of the stormwater volume was bypassed by Harvesting RTC, resulting in a lower total removal rate compared to the biofilters without RTC (62.3 % vs. 93.7 %). While with Harvesting-Environment RTC, the environment was protected no worse than when RTC was not implemented. In addition, Both Harvesting RTC and Harvesting-Environment RTC were beneficial to TN removal in the harvested water; however, neither of them were favoured in TSS or TP removal.

(4) Model modification and validation for real time control, and further exploration of control strategies

After the modification of the faecal microbial removal model developed in Chapter 3, the new model, BioRTC, is capable of simulating the outcome of RTC implementation, as the model prediction fitted the laboratory observation very well, even when the parameters previously calibrated from another system were adopted. With previously calibrated parameter sets, Nash Sutcliffe Efficiency (E_c) ranged from -0.07 ~ 0.70 (0.44 for the previous optimum set). When the model was re-calibrated with the RTC experimental data, a promising

E_c value of 0.80 could be achieved. Therefore, it is feasible to utilise BioRTC for RTC benefits assessment.

Two additional RTC strategies, Modified Harvesting RTC and Harvesting-Environment RTC on biofilters with 1.5 SZ (submerged zone), were conceptualised. Modified Harvesting RTC was built on Harvesting RTC by utilising the spare pore volume in biofilters' unsaturated zone, while Harvesting-Environment RTC on biofilters with 1.5 SZ was based on Harvesting-Environment RTC by increasing the biofilter's submerged zone depth/volume 1.5 times higher.

According to the results of the scenario testing that had a modelled representation of reality as input, both the newly conceptualised RTC strategies could better balance the competing needs in stormwater harvesting and reuse (e.g., large volume and good quality of harvested water). Compared to Harvesting RTC, Modified Harvesting RTC increased the water volume for harvesting and total load removal rate, and the water quality for harvesting only was only negligibly impacted. While when Harvesting-Environment RTC on biofilters with 1.5 SZ was adopted, compared to the results obtained from Harvesting-Environment RTC (on biofilters with 1 SZ) simulation, the volume of water collected for harvesting increased to around 1.5 times higher, and the harvested water was of even higher quality.

The benefits of the same RTC strategy and the corresponding optimum set-points might vary for different systems with different operational conditions (e.g., inflow concentrations and dry lengths) and different treatment requirements (e.g., end uses). As such, the model developed in this Chapter, BioRTC, could be an effective tool to determine the optimum RTC strategy and the corresponding set-points according to the input conditions and end use requirements.

7.3 Conclusions and practical implications

To be more specific, the model developed in this study could be utilised for the assessment of biofilter design in practice, including plant and media selection, and submerged zone depth determination. Also, it could be adopted to evaluate the long-term performance of a newly established system and/or a large scale field system, where the practical monitoring and test is very costly in terms of money, labour and time. Furthermore, when no data are available for a certain system, the parameters determined in another system with a similar design could be simply employed without further calibration.

More importantly, the BioRTC model developed in this study is an effective tool for RTC evaluation and selection, and the parameters calibrated for biofilter without RTC could also be adopted directly for RTC prediction. Although in this study, only four RTC strategies were modelled, it is very easy to employ this model to explore and evaluate other potential RTC strategies.

RTC is an effective tool to achieve operational optimisation of stormwater biofilters to reduce the risks posed by faecal microbes during stormwater harvesting and reuse. The two RTC strategies developed and tested in this study have been effective for stormwater harvesting, and the two newly conceptualised RTC could even better balance the competing needs in stormwater harvesting and reuse. It is noted that, the selection of RTC strategy may vary for different systems and end uses.

Harvesting RTC aims to collect as much harvestable water as possible, with little consideration of environmental protection. One benefit of this strategy is, the water collected for harvesting is of large volume, as most of the “clean” water with sufficient retention time will be collected. Therefore, this strategy is suggested to be adopted when the environmental protection is not the priority while the water volume for harvesting is of major concern.

Modified Harvesting RTC utilises the extra pore volume in USZ (unsaturated zone) to allow more stormwater runoff enters into biofilters. Therefore,

compared to Harvesting RTC, it could provide additional water for harvesting and reduce environmental pollution. It is suggested to apply this strategy when the inflow concentration is low, so that the water quality for harvesting would not be significantly impacted compared to that of Harvesting RTC.

Harvesting-Environment RTC collects harvestable water in advance, and could protect the environment no worse than without implementing RTC. The water collected from Harvesting-Environment RTC for harvesting is of very high quality, however, due to the water holding capacity of the filter media in biofilters, only limited water with sufficient retention time could be collected for harvesting. As such, Harvesting-Environment RTC is highly recommended when high water quality is required but the water volume for harvesting is less important. In addition, although the uncertainties caused by inaccurate rainfall forecast is out of the scope of this study, the source of rainfall forecast information is crucial and should be carefully selected in practical.

Enlarging SZ volume is effective to increase the water volume that collected from Harvesting-Environment RTC biofilters for harvesting and reuse, without jeopardising the harvested water quality. In practice, the SZ volume of biofilters is suggested to be designed as large as possible.

For different systems, the set-points and the outcomes of the same strategy could be various. Therefore, for a certain system, the potential RTC strategies and corresponding set-points are suggested to be tested case by case, and the modelling tool developed in this study, BioRTC, could be employed to determine the optimum RTC strategy and corresponding set-points.

7.4 Strengths and limitations

(1) Modelling of microbial removal in stormwater biofilters

The model developed in Chapter 3 is a rare process-based model for the prediction of microbial removal in stormwater biofilters that could provide a long-term prediction of the removal of faecal microbes. This model is capable of evaluating the performance of biofilters with various design and experience

different operational operations. In addition, the model does not have a complicated structure but has been fully validated with different data sets that collected from lab testing and field monitoring. More importantly, there is no need to re-calibrate the model when it is applied into a new system, since the parameters calibrated for another system with similar plant type and media type could be simply adopted. This is crucial for the assessment of field systems, since field monitoring and field data collection are very costly. With this model, only simple laboratory tests are needed to estimate the parameter values for field system prediction.

Furthermore, after the modification of this model developed in Chapter 3, the new model for RTC simulation, BioRTC (developed in Chapter 5), could serve as a fundamental part to achieve RTC of stormwater biofilters, as it is capable of reflecting the benefits of employing RTC to adjust the operational conditions (e.g., infiltration rate, retention time, and inflow volume) of biofilters with various designs. In addition, this model has been successfully validated with the data collected from laboratory experiments with RTC implementation, further demonstrating that it could be employed to assess different RTC strategies under various scenarios. Moreover, although only off-line strategies (i.e., operational objectives are specified prior to the actual control process, and set-points are normally fixed) have been tested in this study, BioRTC can also be applied in the implementation of on-line strategies (i.e., operational objectives are defined as a mathematical function, and set-points are variable and determined during the control process), as BioRTC has a rather simple structure, is easy to run, and could provide promising prediction promptly.

Although the two models developed in this study have multiple strengths, some limitations exist. For example, the models do not capture some processes that may occur during the operation of stormwater biofilters, such as preferential flow, clogging after long time operation, and cracking of filter media after dry periods (Bright et al., 2010; FAWB, 2009). As such, the performance of the models can be compromised under these circumstances. However, these phenomena are rather random and very hard to describe with deterministic mathematical equations. More importantly, although these rather simple

models do not include these random processes, they could still provide reasonably good predictions for different systems, and have been successfully validated with various data sets.

Another limitation in modelling is that the BioRTC model was only validated using laboratory-collected data for RTC. It would better demonstrate the capability of this model, if BioRTC could also be validated by the data collected from a field system where RTC has been implemented. However, it is important to note that, before modification, the model developed in Chapter 3 has shown good transferability between laboratory and field scale systems that operate under non-RTC conditions (demonstrated in Chapter 4). In addition, BioRTC has the same model structure with the model developed in Chapter 3, and the results in Chapter 6 indicated that even the non-RTC biofilters' parameter values could be utilised in BioRTC for RTC prediction. These evidence suggested that RTC modelling with BioRTC is also very likely to be transferable from laboratory conditions to field conditions.

In addition, only one of the microbial indicators, *E. coli*, was selected to test the model developed in Chapter 3 and the BioRTC model introduced in Chapter 6. How these models would perform in predicting other types of microbes (e.g., enterococci, *Campylobacter* spp.) is still unknown. Importantly, the literature indicates that the governing processes for *E. coli* removal (e.g., adsorption, desorption, straining, and die-off) are also applicable to other types of microbes (although the importance of each individual process may vary for different types of microbes). Therefore, it is expected that the models could still provide a good prediction for other types of microbes, albeit with different calibrated parameter values and perhaps a slightly modified structure.

(2) Experimental tests of RTC

To the author's knowledge, this study initiates the first attempt to develop RTC strategies for stormwater biofilters. The two strategies that developed and tested in Chapter 5, Harvesting RTC and Harvesting-Environment RTC, have been demonstrated to provide great benefits in the laboratory tests. For Harvesting RTC, the harvested water from RTC biofilters for harvesting could

meet the secondary contact for recreational water use. While for Harvesting-Environment RTC, the requirements for dual reticulation with indoor and outdoor use or irrigation of commercial food crops were met in the harvested water of RTC-biofilters. Also, the set-points in these two strategies (e.g., minimum required retention time for submerged zone water) are easy to adjust, ensuring that the strategies are adjustable under different weather conditions (e.g., different rainfall durations and dry lengths between wet events) and inflow conditions (e.g., various inflow volumes and concentrations) to meet the requirements for different end uses.

However, the implementation of RTC in field-scale systems have not been practically conducted in this research, only modelling has been employed for the simulation of scenarios that using a modelled representation of reality. A better understanding of the benefits of RTC might be achieved if field implementation could be conducted with a long-term operation. Importantly, this study is the first study on RTC of stormwater biofilters, and provides the first evidence of how these systems could potentially perform under active, rather than passive, operation.

Since this study mainly focused on the development of the framework rather than to explore methods to achieve control, the control processes were all manually implemented in the laboratory experiments. Therefore, some practical implications during the incorporation of RTC facilities, such as sensors, have not been fully revealed.

In addition, in the laboratory experiments for RTC testing, Harvesting RTC and Harvesting-Environment RTC were tested one after the other (i.e., in separate rounds), but not in parallel. Therefore, bias might occur during the operation of the two rounds experiments, preventing a full comparison between the two strategies. Also, before the start of the laboratory experiments for RTC testing, the biofilter columns had already been operated for approximately three years. Since three years is a rather “old age” of stormwater biofilters, the effects of these two RTC strategies in a typical biofilter might not be fully presented.

Moreover, for Harvesting-Environment RTC, only perfect rainfall forecast information was adopted; that was because, no required historical rainfall prediction data (e.g., 3-hourly weather prediction) were available in the study site during the laboratory experiments. Therefore, the uncertainty introduced by inaccurate rainfall prediction has not been reflected. However, by assuming "perfect" rainfall prediction, the benefits that purely generated from Harvesting-Environment RTC implementation have been revealed.

Furthermore, only off-line RTC strategies have been tested in this study, and no on-line RTC strategy has been developed or tested. Therefore, how would an on-line RTC strategy for stormwater biofilters look like and what its effect would be is not unknown. However, it is also important to notice that, the off-line RTC strategies developed and tested in this study have already been proved very effective.

Lastly, this study mainly focused on the removal of microbes in stormwater, with few tests were placed the removal of TSS (total suspended solids), TN (total nitrogen) and TP (total phosphorus). Both positive and negative effects have been found for the removal of TSS, TN, and TP during RTC implementation. Therefore, more studies are suggested to (1) reveal the impacts of RTC that developed for stormwater harvesting and reuse on other pollutants, such as micro-pollutants and heavy metals; and (2) develop exclusive RTC strategies for other pollutants to broad the application of RTC in fulfilling different treatment objectives.

7.5 Future work

Future work aims to eliminate the limitations as stated above, and to provide more profound research outputs in RTC of stormwater biofilters.

(1) Further testing of microbial removal model

As aforementioned, in this study, both the developed microbial removal model developed in Chapter 3 and the BioRTC model introduced in Chapter 6 were only employed for *E. coli* prediction, and the models' performance in predicting

other types of microbes (such as enterococci and *Campylobacter* spp.) needs to be tested, to ensure that these models have a broad application. Therefore, these models will tested with the data collected from the experiments on other microbes in the future.

In addition, neither of the two models could capture some processes that may cause uncertainties in prediction, such as preferential flow, and clogging and cracking of filter media. Therefore, it would improve the performance of current models if these processes could be reasonably interpreted. Although a few models have been developed to describe similar processes in the field of underground water (Dingman, 2002), the number of parameters should also be limited to prevent over-parameterisation. In addition, extra computation burden should also be avoided, otherwise the application of the model in RTC might be confined. Considering these, to develop simple empirical model components and incorporated with current models could be an option to solve this problem. However, to achieve this aim, additional data need to be collected and analysed.

(2) Field implementation of RTC strategies

In this study, the effectiveness of RTC in field systems has not been tested. The weather conditions (e.g., the length of dry period and rainfall duration) and inflow conditions (e.g., inflow volumes and concentrations) in a field system would be very different to those in a lab-scale systems; especially, extreme conditions (e.g., extended dry periods or extremely high inflow volume) are more likely to occur in the field. Therefore, field implementation of RTC is necessary for future work. Field testing of RTC could provide field monitoring data to better validate the model for RTC prediction, and better reveal the benefits of RTC in field application under extreme conditions.

In addition, field testing could reflect the functions and uncertainties caused by the application of RTC facilities, such as the failure of sensors and uncertainties introduced by rainfall forecasting. It is expected that both failure of sensors and rainfall forecasting could negatively affect RTC's effectiveness: the former may cause that the control actions fail to be implemented, while the latter may result in the implementation of inappropriate actions.

Furthermore, Field testing could help to gather experience in maintenance, as maintenance governs the effectiveness of biofilters, according to many studies and practical experiences. Although the implementation of RTC may require additional maintenance on control facilities (e.g., sensors, valves, and flowmeters), it is expected that RTC could help to enhance the maintenance quality for the entire system, so that the effectiveness of biofilters could be improved. This assumption is based on the fact that, RTC facilities could monitor what occurs within biofilters, and this information could lead to a more efficient maintenance. For example, a moisture sensor could help to detect the occurrence of clogging, so that prompt maintenance could be conducted.

(3) Further development and testing of RTC strategies

In this study, three RTC strategies have been developed and tested through modelling, and two of them were tested with laboratory experiments. However, there are also other potential RTC strategies for stormwater biofilters, such as decreasing the infiltration rates in the outflow at the end of rainfall to increase retention time (Chandrasena et al., 2014b). Besides, the further development of RTC may also result in the innovation the biofilters' design: e.g., the raised outflow pipe in a traditional biofilter might be able to be eliminated, as the submerged zone depth could be controlled by a valve in the bottom pipe and adjusted by opening/closing the valve according to instantaneous operational conditions; as such, "novel" biofilters might be designed to better incorporate RTC strategies in the future.

In addition, the strategy presented in this study mainly focused on the removal of microbes for stormwater harvesting and reuse, further strategies may consider to balance it with nutrient removal. To achieve this, one potential method is to keep the retention time neither too long (not favoured in nutrient removal) nor too short (not favoured in microbial removal).

Moreover, since only off-line strategies have been tested in this study, future work will focus more on on-line strategies, with the help of modelling and monitoring facilities (e.g., sensors).

(4) RTC simulation and benefits evaluation for different catchments in different cities and countries

In this study, only one catchment in Melbourne, Australia was selected in the tested scenarios. However, for different catchments in different cities, the benefits of RTC would be various, as the weather conditions (e.g., antecedent dry days, rainfall frequency), and inflow rates and microbial concentrations may vary. These differences may impact strategy selection as well as the set-points selection of RTC. A systematic test of some typical cities in Australia and other countries would be necessary to provide a comprehensive evaluation of RTC.

(5) Searching for a surrogate as the microbial concentration indicator

At this moment, online measurement of microbial concentration could hardly be achieved; therefore, only offline RTC strategies have been tested in this study. In the future, if a surrogate could be found as microbial concentration indicator to achieve online monitoring, more efficient RTC strategies could be developed and RTC strategies could even be adjusted during an event. Turbidity might be a potential surrogate during some stages of operation, as microbes could be associated with soil that flushed out from biofilters. Some turbidity data have been collected during the laboratory experiments in this study, however, thorough data analysis and more data collection need to be conducted.

(6) Evaluation of cost-benefits of RTC adoption

In this study, it is concluded that enlarging SZ volume/depth is effective to increase the benefits of adopting Harvesting-Environment RTC. However, this method will also increase the cost in practical. Therefore, cost-benefit analysis, such as generating a curve on the benefits of different SZ sizes against the corresponding cost would provide more evidence for biofilter design selection in practical implementation.

7.6 References

Alexander M. Why microbial predators and parasites do not eliminate their prey and hosts. Annual Review of Microbiology 1981; 35: 113-133.

Allen R. G., Pereira L. S., Raes D., Smith M. Corp evapotranspiration - Guidelines for computing crop water requirements - FAO Irrigation and

Drainage Paper No.56. FAO - Food and Agriculture Organization of the United Nations, Rome, Italy, 1998.
APHA-AWWA-WEF. Standard Methods for the Examination of Water and Wastewater, the 21st. Edition: American Public Health Association, Washington, 2005.
Arnone R. D., Walling J. P. Waterborne pathogens in urban watersheds. Journal of Water and Health 2007; 5: 149-162.
Bales R. C., Hinkle S. R., Kroeger T. W., Stocking K., Gerba C. P. Bacteriophage adsorption during transport through porous media: Chemical perturbations and reversibility. Environmental Science & Technology 1991; 25: 2088-2095.
Beven K., Binley A. The future of distributed models: Model calibration and uncertainty prediction. Hydrological Processes 1992; 6: 279-298.
Bitton G., Gerba C. P. Microbial Pollutants: Their Survival and Transport Pattern to Groundwater. Groundwater Pollution Microbiology. John Wiley and Sons, New York, USA, 1984.
Boyd J. W., Yoshida T., Vereen L. E., Cada R. L., Morrison S. M. Bacterial Response to the Soil Environment. Colorado Water Resources Research Institute, Colorado State University, Fort Collins, Colorado, 1969.
Bradford S. A., Simunek J., Walker S. L. Transport and straining of *E. coli* O157:H7 in saturated porous media. Water Resources Research 2006; 42: W12S12.
Bratieres K., Fletcher T. D., Deletic A., Zinger Y. Nutrient and sediment removal by stormwater biofilters: A large-scale design optimisation study. Water Research 2008; 42: 3930-3940.
Brauwere A. d., Ouattara N. K., Servais P. Modeling Fecal Indicator Bacteria Concentrations in Natural Surface Waters: A Review. Critical Reviews in Environmental Science and Technology 2014; 44: 2380-2453.
Bright T., Hathaway J., Hunt W., III, de los Reyes F., III, Burchell M., II. Impact of storm-water runoff on clogging and fecal bacteria reduction in sand columns. Journal of Environmental Engineering 2010; 136: 1435-1441.
Brookes J., Hipsey M., Burch M. D., Linden L. G., Ferguson C., Antenucci J. Relative value of surrogate indicators for detecting pathogens in lakes and reservoirs. Environmental Science & Technology 2005; 39: 8614-8621.
Brookes J. D., Antenucci J., Hipsey M., Burch M. D., Ashbolt N. J., Ferguson C. Fate and transport of pathogens in lakes and reservoirs. Environment International 2004; 30: 741-759.
Brown R. A., Hunt W. F. Improving bioretention/biofiltration performance with restorative maintenance. **Water Science & Technology** 2012; 65: 361-367.
Burton G. A., Gunnison D., Lanza G. R. Survival of pathogenic bacteria in various freshwater sediments. Applied and Environmental Microbiology 1987; 53: 633-638.
Burton G. A., Pitt R. E. Stormwater effects handbook: a toolbox for watershed managers, scientists and engineers. Boca Raton, Florida: Lewis Publishers, 2002.
Caramia M., Dell'Olmo P. Multi-objective Management in Freight Logistics - Increasing Capacity, Service Level and Safety with Optimization Algorithms: Springer-Verlag London, 2008.

Carleton J. N., Grizzard T. J., Godrej A. N., Post H. E., Lampe L., Kenel P. P. Performance of constructed wetlands in treating urban stormwater runoff. Water Environment Research 2000; 72: 295-304.

Chandrasena G. I. Faecal Microorganism Removal in Stormwater Biofilters. Department of Civil Engineering. Doctor of Philosophy. Monash University, 2014.

Chandrasena G. I., Deletic A., A. L., Henry R., McCarthy D. T. Stormwater biofilters as barriers against *Campylobacter jejuni*, *Cryptosporidium* oocysts and adenoviruses; results from a laboratory trial. Water 2017; 9: 949.

Chandrasena G. I., Deletic A., Ellerton J., McCarthy D. T. Evaluating *Escherichia coli* removal performance in stormwater biofilters: a laboratory-scale study. Water Science & Technology 2012; 66: 1132-1138.

Chandrasena G. I., Deletic A., McCarthy D. T. Evaluating *Escherichia coli* removal performance in stormwater biofilters: a preliminary modelling approach. Water Science & Technology 2013; 67: 2467-75.

Chandrasena G. I., Deletic A., McCarthy D. T. Survival of *Escherichia coli* in stormwater biofilters. Environmental Science and Pollution Research 2014a; 21: 391-401.

Chandrasena G. I., Deletic A., McCarthy D. T. Biofiltration for stormwater harvesting: Comparison of *Campylobacter spp.* and *Escherichia coli* removal under normal and challenging operational conditions. Journal of Hydrology 2016; 537: 248-259.

Chandrasena G. I., Pham T., Payne E. G., Deletic A., McCarthy D. T. *E. coli* removal in laboratory scale stormwater biofilters: Influence of vegetation and submerged zone. Journal of Hydrology 2014b; 519: 814-822.

Chang L.-C., Chang F.-J. Intelligent control for modelling of real-time reservoir operation. Hydrological Processes 2001; 15: 1621-1634.

Cizek A. R., Characklis G. W., Krometis L. A., Hayes J. A., Simmons III O. D., Di Lonardo S., et al. Comparing the partitioning behavior of Giardia and Cryptosporidium with that of indicator organisms in stormwater runoff. Water Research 2008; 42: 4421-4438.

Corominas L., Sin G., Puig S., Traore A., Balaguer M., Colprim J., et al. Model-based evaluation of an on-line control strategy for SBRs based on OUR and ORP measurements. Water Science & Technology 2006; 53: 161.

Coustumer S. L., Fletcher T. D., Deletic A., Barraud S., Poelsm P. The influence of design parameters on clogging of stormwater biofilters: A large-scale column study. Water Research 2012; 46: 6743–6752.

Crane S. R., Moore J. A. Modeling Enteric Bacterial Die-off - A Review. Water, Air, and Soil Pollution 1986; 27: 411-439.

Criss R. E., Winston W. E. Do Nash values have value? Discussion and alternate proposals. Hydrological Processes 2008; 22: 2723-2725.

CSIRO. Urban stormwater best practice environmental management guidelines (BPEMG). Commonwealth Scientific and Industrial Research Organisation (CSIRO), Victoria, Australia, 1999.

CWSC. Project 1: Sustainable Technologies. Centre for water sensitive cities (CWSC), Australia, 2010.

Daly E., Bach P. M., Deletic A. Stormwater pollutant runoff: a stochastic approach. Advances in Water Resources 2014; 74: 148-155.
Davies C. M., Bavor H. J. The fate of stormwater associated bacteria in constructed wetland and water pollution control pond systems. Journal of Applied Microbiology 2000; 89: 349-360.
Davies C. M., Yousefi Z., Bavor H. J. Occurrence of coliphages in urban stormwater and their fate in stormwater management systems. Letters in Applied Microbiology 2003; 37: 299-303.
DHV. Guidelines for validating treatment processes for pathogen reduction: Supporting Class A recycled water schemes in Victoria. Department of Health, Victoria, 2013.
Díaz F. J., O'Geen A. T., Dahlgren R. A. Efficacy of Constructed Wetlands for Removal of Bacterial Contamination From Agricultural Return Flows. Agricultural Water Management 2010; 97: 1813-1821.
Dingman S. L. Physical Hydrology: Upper Saddle River, N.J. : Prentice Hall, 2002.
Dotto C. B. S., Mannina G., Kleidorfer M., Vezzaro L., Henrichs M., McCarthy D. T., et al. Comparison of different uncertainty techniques in urban stormwater quantity and quality modelling. Water Research 2012; 46: 2545-2558.
Duncan H. P. Urban Stormwater Quality: A Statistical Overview. Melbourne, Australia: Cooperative Research Centre for Catchment Hydrology, 1999.
Ecowise. Characterisation of human health risk derived from stormwater. Ecowise Environmental PTY Ltd., 2010.
Edberg S. C., Rice E. W., Karlin R. J., Allen M. J. Escherichia coli: The best biological drinking water indicator for public health protection. Journal of Applied Microbiology Symposium Supplement 2000; 88: 106s-116s.
FAWB. Adoption Guidelines for Stormwater Biofiltration Systems. Facility for Advancing Water Biofiltration, Monash University, 2009.
Feng W., Hatt B. E., McCarthy D. T., Flecther T. D., Deletic A. Biofilters for stormwater harvesting: Understanding the treatment performance of key metals that pose a risk for water use. Environmental Science & Technology 2012; 46: 5100-5108.
Ferguson C., De Roda Husman A. M., Altavilla N., Deere D., Ashbolt N. Fate and transport of surface water pathogens in watersheds. Critical Reviews in Environmental Science and Technology 2003; 33: 299-361.
Fletcher T. D., Deletic A., Mitchell V. G., Hatt B. Reuse of urban runoff in Australia: a review of recent advances and remaining challenges. Journal of Environmental Quality 2008; 37: 116 - 127.
Fletcher T. D., Mitchell V. G., Deletic A., Ladson A. Is stormwater harvesting beneficial to urban waterway flow? Water Science & Technology 2007; 55: 265-272.
Foppen J. W., van Herwerden M., Schijven J. Measuring and modelling straining of *Escherichia coli* in saturated porous media. Journal of Contaminant Hydrology 2007; 93: 236-54.
Fowdar H. S., Hatt B. E., Breen P., Cook P. L. M., Deletic A. Designing living walls for greywater treatment. Water Research 2017; 110: 218-232.

Franchi A., O'Melia C. R. Effects of natural organic matter and solution chemistry on the deposition and reentrainment of colloids in porous media. Environmental Science & Technology 2003; 37: 1122-1129.
Gaborit E., Muschalla D., Vallet B., Vanrolleghem P. A., Anctil F. Improving the performance of stormwater detention basins by real-time control using rainfall forecasts. Urban Water Journal 2013; 10: 230-246.
Gargiulo G., Bradford S., Šimůnek J., Ustohal P., Vereecken H., Klumpp E. Bacteria transport and deposition under unsaturated conditions: the role of the matrix grain size and the bacteria surface protein. Journal of Contaminant Hydrology 2007; 92: 255-273.
Gargiulo G., Bradford S. A., Simunek J., Ustohal P., Vereecken H., Klumpp. E. Bacteria Transport and Deposition under Unsaturated Flow Conditions: The Role of Water Content and Bacteria Surface Hydrophobicity. Vadose Zone Journal 2008; 7: 406-419.
Garzio-Hadzick A., Shelton D. R., Hill R. L., Pachepsky Y. A., Guber A. K., Rowland R. Survival of manure-borne E. coli in streambed sediment: effects of temperature and sediment properties. Water Research 2010; 44: 2753-2762.
Göbel P., Dierkes C., Coldewey W. G. Storm water runoff concentration matrix for urban areas. Journal of Contaminant Hydrology 2007; 91: 26-42.
Han H., Qiao J. Nonlinear Model-Predictive Control for Industrial Processes: An Application to Wastewater Treatment Process. IEEE TRANSACTIONS ON INDUSTRIAL ELECTRONICS 2014; 61: 1970-1980.
Harter T., Wagner S., Atwill E. R. Colloid transport and filtration of Cryptosporidium parvum in sandy soils and aquifer sediments. Environmental Science & Technology 2000; 34: 62-70.
Hathaway J., Hunt W. Indicator Bacteria Removal by Stormwater BMPs in Coastal North Carolina. World Environmental and Water Resources Congress: Challenges of Change, Providence, Rhode Island, United States, 2010, pp. 3218-3225.
Hathaway J., Hunt W., Graves A., Wright J. Field Evaluation of Bioretention Indicator Bacteria Sequestration in Wilmington, North Carolina. Journal of Environmental Engineering 2011; 137: 1103-1113.
Hathaway J. M., Hunt W. F., Jadlocki S. Indicator bacteria removal in storm-water best management practices in charlotte, north carolina. Journal of Environmental Engineering 2009; 135: 1275-1285.
Hatt B. E., Deletic A., Fletcher T. D. Integrated Stormwater Treatment and Re-use Systems - Inventory of Australian practice. Cooperative Research Centre for Catchment Hydrology, Melbourne, Australia, 2004.
Hatt B. E., Deletic A., Fletcher T. D. Integrated treatment and recycling of stormwater: a review of Australian practice. J Environ Manage 2006; 79: 102-13.
Hatt B. E., Fletcher T. D., Deletic A. Hydraulic and pollutant removal performance of stormwater filters under variable wetting and drying regimes. Water Science & Technology 2007; 56: 11-19.
Hatt B. E., Fletcher T. D., Deletic A. Hydrologic and pollutant removal performance of stormwater biofiltration systems at the field scale. Journal of Hydrology 2009; 365: 310-321.

Haznedaroglu B. Z., Kim H. N., Bradford S. A., Walker S. L. Relative transport behavior of Escherichia coli O157:H7 and Salmonella enterica serovar pullorum in packed bed column systems: Influence of solution chemistry and cell concentration. Environmental Science & Technology 2009; 43: 1838-1844.

Hijnen W. A. M., Brouwer-hanzens A. J., Charles K. J., Medema G. J. Transport of MS2 phage, Escherichia coli Clostridium perfringens, Cryptosporidium parvum, and Giardia intestinalis in a gravel and a sandy soil. Environmental Science and Technology 2005; 39: 7860-7868.

Hipsey M. R., Antenucci J. P., Brookes J. D. A generic, process-based model of microbial pollution in aquatic systems. Water Resources Research 2008; 44.

Horan N. J. Faecal indicator organisms. The Handbook of Water and Wastewater Microbiology. Academic Press, United Kingdom, 2003.

Hsu N.-S., Huang C.-L., Wei C.-C. Multi-phase intelligent decision model for reservoir real-time flood control during typhoons. Journal of Hydrology 2015; 522: 11-34.

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

Johnson W. P., Zhang P., Gradner P. M., Fuller M. E., Deflaun M. F. Modeling bacterial detachment during transport through porous media as a residence-time-dependent process. Applied and Environmental Microbiology 1995; 67: 4908-4913.

Jones M. P., Hunt W. F. Bioretention impact on runoff temperature in trout sensitive waters. Journal of Environmental Engineering 2009; 135: 577-585.

Jovanovic D., Hathaway J., Coleman R., Deletic A., McCarthy D. T. Conceptual modelling of E. coli in urban stormwater drains, creeks and rivers. Journal of Hydrology 2017; 555: 129-140.

Jung J., Fowdar H., Henry R., Deletic A., McCarthy D. Biofilters as effective pathogen barriers for greywater reuse. Ecological Engineering 2019: under review.

Kadlec R. H., Wallace S. Treatment wetlands (Second edition): CRC Press, 2008.

Kandra H. S., McCarthy D., Fletcher T. D., Deletic A. Assessment of clogging phenomena in granular filter media used for stormwater treatment. Journal of Hydrology 2014; 512: 518-527.

Kerkez B., Gruden C., Lewis M., Montestruque L., Quigley M., Wong B., et al. Smarter Stormwater Systems. Environmental Science & Technology 2016; 50: 7267-7273.

Kirk R. E. Introduction to the Analysis of Variance. Statistics: An Introduction (5th edition). Thomson/Wadsworth, Belmont, CA, 2008, pp. 392-429.

Krometis L.-A. H., Drummey P. N., Characklis G. W., Sobsey M. D. Impact of microbial partitioning on wet retention pond effectiveness. Journal of Environmental Engineering 2009; 135: 758-767.

Langeveld J. G., Benedetti L., de Klein J. J. M., Nopens I., Amerlinck Y., van Nieuwenhuijzen A., et al. Impact-based integrated real-time control for improvement of the Dommel River water quality. Urban Water Journal 2013; 10: 312-329.

Le Coustumer S., Fletcher T. D., Deletic A., Barraud S., Poelsma P. The influence of design parameters on clogging of stormwater biofilters: a large-scale column study. Water Research 2012; 46: 6743-52.

Leon A. S., Kanashiro E. A., Valverde R., Sridhar V. Dynamic Framework for Intelligent Control of River Flooding: Case Study. Journal of Water Resources Planning and Management 2014; 140: 258-268.

Li Y., McCarthy D. T., Deletic A. *Escherichia coli* removal in copper-zeolite-integrated stormwater biofilters: Effect of vegetation, operational time, intermittent drying weather. Ecological Engineering 2016; 90: 234-243.

Li Y. L., Deletic A., Alcazar L., Bratieres K., Fletcher T. D., McCarthy D. T. Removal of Clostridium perfringens, Escherichia coli and F-RNA coliphages by stormwater biofilters. Ecological Engineering 2012; 49: 137-145.

Lim C. H., Flint K. P. The effect of nutrients on the survival of Escherichia coli in lake water. Journal of Applied Bacteriology 1989; 66: 559-569.

Lund N. S. V., Falk A. K. V., Borup M., Madsen H., Mikkelsen P. S. Model predictive control of urban drainage systems: A review and perspective towards smart real-time water management. Critical Reviews in Environmental Science and Technology 2018; 48: 279-339.

Makepeace D. K., Smith D. W., Stanley S. J. Urban stormwater quality: Summary of contaminant data. Critical Reviews in Environmental Science and Technology 1995; 25: 93-139.

Malaviya P., Singh A. Constructed Wetlands for Management of Urban Stormwater Runoff. Critical Reviews in Environmental Science and Technology 2012; 42: 2153-2214.

Mallin M. A., Ensign S. H., Wheeler T. L., Mayes D. B. Pollutant removal efficacy of three wet detention ponds. Journal of Environmental Quality 2002; 31: 654-660.

McCarthy D. T., Deletic A., Mitchell V. G., Diaper C. Development and testing of a model for Micro-Organism Prediction in Urban Stormwater (MOPUS). Journal of Hydrology 2011; 409: 236-247.

McCarthy D. T., Deletic A., Mitchell V. G., Fletcher T. D., Diaper C. Uncertainties in stormwater E. coli levels. Water Research 2008; 42: 1812-1824.

Meneses E. J., Gaussens M., Jakobsen C., Mikkelsen P. S., Grum M., Vezzaro L. Coordinating Rule-Based and System-Wide Model Predictive Control Strategies to Reduce Storage Expansion of Combined Urban Drainage Systems: The Case Study of Lundtofte, Denmark. Water 2018; 10: 15.

Meng Z., Chandrasena G., Henry R., Deletica A., Kolotelo P., McCarthy D. Stormwater constructed wetlands: A source or a sink of *Campylobacter* spp. Water Research 2018; 131: 218-227.

Mitchell V. G., D.T. McCarthy, Deletic A., Fletcher T. D. Urban stormwater harvesting e sensitivity of a storage behaviour model. Environmental Modelling & Software 2008; 23: 782-793.

Mitchell V. G., Deletic A., Fletcher T. D., Hatt B. E., McCarthy D. T. Achieving multiple benefits from stormwater harvesting. Water Science & Technology 2007; 55: 135-144.

Mukerji K. G., Manoharachary C., Singh J. Microbial Activity in the Rhizosphere. Berlin: Berlin Springer, 2006.

Mullapudi A., Bartos M., Wong B., Kerkez B. Shaping Streamflow Using a Real-Time Stormwater Control Network. Sensors 2018; 18: 2259.
Murphy H. M., Meng Z., Henry R., Deletic A., McCarthy D. T. Current Stormwater Harvesting Guidelines Are Inadequate for Mitigating Risk from *Campylobacter* During Nonpotable Reuse Activities. Environmental science & technology 2017; 51: 12498-12507.
NHMRC. Australian Drinking Water Guidelines. Australian Government, National Health and Medical Research Council, 2004.
NHMRC. Guidelines for Managing Risks in Recreational Water. Australian Government, National Health and Medical Research Council, Canberra, Australia, 2008.
NHMRC. Australian Guidelines for Water Recycling (Phase 2). Stormwater Harvesting and Reuse. Australian Government, Natural Resource Management Ministerial Council, Environment Protection and Heritage Council, National Health and Medical Research Council, Canberra, Australia, 2009.
Oliveri V. P., Kruse C. W., Kawata K. Microorganisms in Urban Stromwater (EPA-600/2-77-087). US EPA, 1977.
Pachepsky Y. A., Shelton D. R. Escherichia Coliand Fecal Coliforms in Freshwater and Estuarine Sediments. Critical Reviews in Environmental Science and Technology 2011; 41: 1067-1110.
Pareto V. The New Theories of Economics. Journal of Political Economy 1897; 5: 485-502.
Payne E. G. I., Pham T., Cook P. L. M., Fletcher T. D., Hatt B. E., Deletic A. Biofilter design for effective nitrogen removal from stormwater – influence of plant species, inflow hydrology and use of a saturated zone. Water Science & Technology 2014; 69: 1312-1319.
Perdek J. M., Arnone R. D., Stinson M. K., Tuccillo M. E. Managing Urban Watershed Pathogen Contamination. U.S. Environmental Protection Agency, Cincinnati, Ohio, 2003.
Reddy K. R., Khaleel R., Overcash M. R. Behavior and transport of microbial pathogens and indicator organisms in soils treated with organic wastes. Journal of Environmental Quality 1981; 10: 255-266.
Redman J. A., Walker S. L., Elimelech M. Bacterial Adhesion and Transport in Porous Media: Role of the Secondary Energy Minimum. Environmental Science & Technology 2004; 38: 1777-1785.
Rusciano G. M., Obropta C. C. Bioretention column study: Fecal coliform and total suspended solids reductions. Transactions of the ASABE 2007; 50: 1261-1269.
Schijven J. F., Hassanizadeh S. M. Removal of viruses by soil passage: Overview of modeling, processes, and parameters. Critical Reviews in Environmental Science and Technology 2000; 30: 49-127.
Schilling W., Andersson B., Nyberg U., Aspegren H., Rauch W., Harremoës P. Real time control of wastewater systems. Journal of Hydraulic Research 1996; 34: 785-797.
Schultz N., Meagher D., Cabibel B. Milwaukee's Experience in Collection System Controls. Proceedings of Water Environment Federation Specialty Conference 2001, 2001.
Schütze M., Butler D., Beck B. M. Modelling, Simulation and Control of Urban Wastewater Systems: Springer Science & Business Media, 2002.

Schütze M., Campisano A., Colas H., Schilling W., Vanrolleghem P. A. Real time control of urban wastewater systems—where do we stand today? Journal of Hydrology 2004; 299: 335-348.

Shen P., Deletic A., McCarthy D. T. Real time control of biofilters delivers stormwater suitable for harvesting and reuse Water Research (submitted) 2018a.

Shen P., Deletic A., Urich C., Chandrasena G. I., McCarthy D. T. Stormwater Biofilter Treatment Model for Faecal Microorganisms. Science of the Total Environment 2018b; 630: 992-1002.

Shen P., McCarthy D. T., Chandrasena G. I., Li Y., Deletic A. Validation and Uncertainty Analysis of a Stormwater Biofilter Treatment Model for Faecal Microorganisms. 2018c.

State of Victoria D. o. S. a. E., Australia. Using the integrated water management provisions of Clause 56 - Residential subdivision, Victoria, Australia, 2006.

Stenstrom T. A., Carlander A. Occurrence and die-off of indicator organisms in the sediment in two constructed wetlands. Water Science & Technology 2001; 44: 223-230.

Stevik T. K., Aa K., Ausland G., Hanssen J. F. Retention and removal of pathogenic bacteria in wastewater percolating through porous media: a review. Water Research 2004; 38: 1355-1367.

Stinson M. Benefits of Sewerage System Real-Time Control. World Water and Environmental Resources Congress 2005, Anchorage, Alaska, United States, 2005, pp. 1-11.

Tian X., van Overloop P.-J., Negenborn R. R., van de Giesen N. Operational flood control of a low-lying delta system using large time step Model Predictive Control. Advances in Water Resources 2015; 75: 1-13.

Tong M., Johnson W. P. Excess Colloid Retention in Porous Media as a Function of Colloid Size, Fluid Velocity, and Grain Angularity. Environmental Science & Technology 2006; 40: 7725-7731.

Tufenkji N. Modeling microbial transport in porous media: Traditional approaches and recent developments. Advances in Water Resources 2007; 30: 1455-1469.

Tufenkji N., Elimelech M. Correlation equation for predicting single-collector efficiency in physicochemical filtration in saturated porous media. Environmental Science & Technology 2004; 38: 529-536.

Uusitalo L., Lehikoinen A., Helle I., Myrberga K. An overview of methods to evaluate uncertainty of deterministic models in decision support. Environmental Modelling & Software 2015; 63: 24-31.

Vandenhove H., Merckx R., Wilmots H., Vlassak K. Survival of Pseudomonas-Fluorescens Inocula of Different Physiological Stages in Soil. Soil Biology & Biochemistry 1991; 23: 1133-1142.

Vezzaro L., Mikkelsen P. S., Deletic A., McCarthy D. Urban drainage models - simplifying uncertainty analysis for practitioners. Water Science & Technology 2013; 68: 2136-43.

Wadzuk B. M., Rea M., Woodruff G., Flynn K., Traver R. G. Water-quality performance of a constructed stormwater wetland for all flow conditions. Journal of the American Water Resources Association 2010; 46: 385-394.

WERF. Development of a Protocol for Risk Assesment of Microorganisms in Seperate Stormwater Systems. Water Environment Research Foundation, 2007.

Willey J. M., Sherwood L. M., Woolverton C. J. Prescott's Microbiology (8th Edition). New York, USA: McGraw-Hill, 2011.

Wong T. H. F. Australian runoff quality: a guide to water sensitive urban design. Crows Nest, N.S.W., Australia: Engineers Australia, 2006.

Wood J., Kelly T., Moran M. Introduction to Urban Stormwater Management in Australia. Department of the Environment and Heritage, Australia, 2002.

Yang Q., Peng Y., Liu X., Zeng W., Mino T., Satoh H. Nitrogen Removal via Nitrite from Municipal Wastewater at Low Temperatures using Real-Time Control to Optimize Nitrifying Communities. Environmental Science & Technology 2007; 41: 8159-8164.

Yates M. V., Yates S. R. Modeling microbial fate in the subsurface environment. 1987; 17: 307-344.

Zhang K., Randelovic A., Deletic A., Page D., T.McCarthy D. Stormwater biofilters: A new validation modelling tool. Ecological Engineering 2016; 87.

Zhang L., Seagren E. A., Davis A. P., Karns J. S. The Capture and Destruction of *Escherichia coli* from Simulated Urban Runoff Using Conventional Bioretention Media and Iron Oxide-coated Sand. Water Environment Research 2010; 82: 701-714.

Zhang L., Seagren E. A., Davis A. P., Karns J. S. Long-term sustainability of *Escherichia coli* removal in conventional bioretention media. Journal of Environmental Engineering 2011; 137: 669-677.

Zhang L., Seagren E. A., Davis A. P., Karns J. S. Effects of temperature on bacterial transport and destruction in bioretention media: Field and laboratory evaluations. Water Environment Research 2012; 84: 485-496.

Zinger Y., Blecken G.-T., Fletcher T. D., Viklander M., Deletić A. Optimising nitrogen removal in existing stormwater biofilters: Benefits and tradeoffs of a retrofitted saturated zone. Ecological Engineering 2013; 51: 75-82.

Zinger Y., Deletic A., Fletcher T. D., Breen P., Wong T. A Dual-mode Biofilter System: Case study in Kfar Sava, Israel. 12th International Conference on Urban Drainage, Porto Alegre, Brazil, 2011.