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Removal of *Clostridium perfringens*, *Escherichia coli* and F-RNA coliphages by stormwater biofilters

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ABSTRACT

Biofiltration is a technology applied to treat urban stormwater runoff that transports various pollutants, including pathogens. However, the pathogen removal performance of biofiltration systems remains unknown. A laboratory study was conducted to investigate the removal of three indicator organisms (*Clostridium perfringens*, *Escherichia coli*, and F-RNA coliphages) by biofilters. The influence of a range of factors was investigated: presence of vegetation, depth and types of filter media, presence of a saturated zone at the base of the biofilter, and intermittent wetting and drying conditions. The mean removal of *C. perfringens* and F-RNA coliphages by all biofilter designs exceeded 3 log. *E. coli* removal during wet periods, however, was much lower (mean 2 log). Furthermore, antecedent drying decreased the *E. coli* removal efficiency significantly ($p < 0.05$). Drying might induce fine fissures or macropore formation in filter media thus reduced retention of microbes. This effect may be more obvious in vegetated designs due to evapotranspiration induced moisture loss. Introducing a saturated zone and carbon source at the base of the filter eliminated such negative effects of drying on *E. coli* removal. The effluent from biofilters with a saturated zone and carbon source met the recommended water quality for secondary contact recreational water use in relation to *E. coli*.

1. Introduction

Untreated urban stormwater runoff has frequently been identified as a nonpoint source of pathogens for many receiving water bodies (USEPA, 2005; Collins et al., 2010), with the potential to adversely affect drinking water sources, recreational waters and aquaculture. Adverse health effects, even waterborne diseases in human populations, have been associated with contacting such contaminated water bodies (Haile et al., 1999; Gaffield et al., 2003). Consequently, urban stormwater management, particularly reduction of pathogen levels, has drawn extensive attention.

For ease of water quality management, *Clostridium perfringens* spores (*C. perfringens*), *Escherichia coli* (*E. coli*), and F-RNA coliphages are commonly used indicators for protozoan, bacterial, and viral pathogens respectively (Ferguson et al., 2003). All these organisms are relatively safe to use and easily detected, thus presenting affordable methods of understanding pathogen behaviour. More importantly, these organisms have shown close relationship with their indicated pathogens in terms of presence, survival, and rare extensive growth in aquatic or soil environment. The main criticism of the use of *C. perfringens* spores as a faecal indicator is its prolonged persistence in the environment (Medema et al., 1997). However, this is one of the reasons it is often used as a conservative indicator for the presence and fate of protozoa in aquatic systems (Payment and Franco, 1993). *E. coli* is a commonly used indicator for assessing overall water quality. F-RNA coliphages were chosen as viral indicators, since they behave relatively conservatively, and have been shown to be very persistent. Moreover, they resemble human enteric viruses in size, shape and composition (Havelaar et al., 1991, 1993; Schijven and Hassanizadeh, 2000).

Biofilters, also known as bioretention systems or rain gardens (Fig. 1), are employed globally to treat urban runoff prior to discharge. Typical biofilters are a combination of natural and engineered systems, which work by filtering stormwater through vegetated filter media that removes pollutants by means of biological uptake, straining and adsorption (Rusciano and Obropta, 2007). Biofilters have been designed and thoroughly studied to remove typical pollutants from stormwater, such as sediments, nutrients

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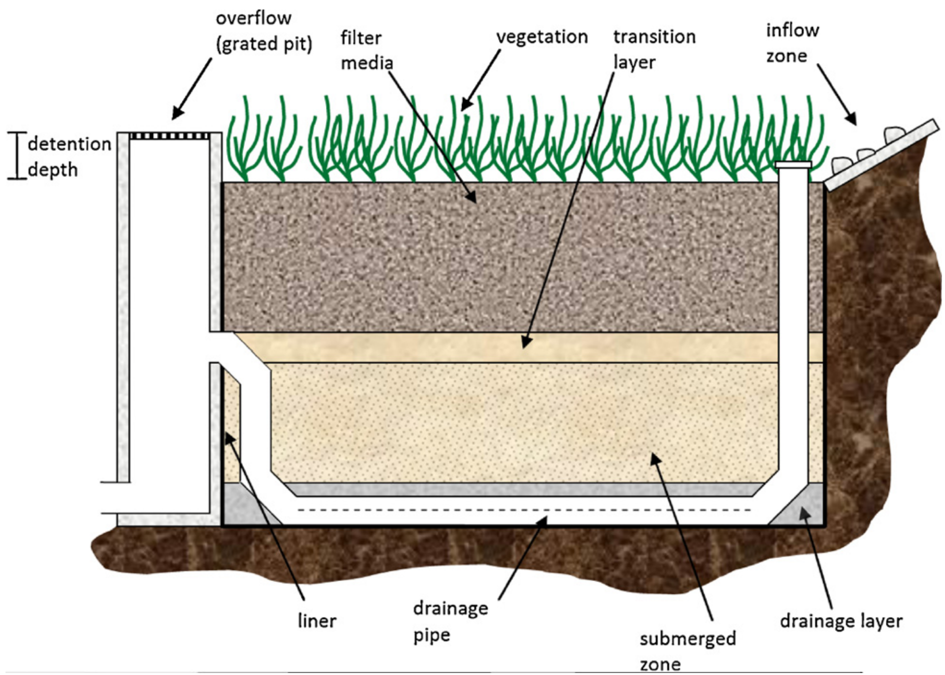

Fig. 1. Biofilter design with saturated zone as recommended by Facility for Advancing Water Biofiltration (FAWB, 2009).

and metals (Davis et al., 2001; Dietz and Clausen, 2006; Dietz, 2007; Fletcher et al., 2008). Yet, their capacity to remove indicator organisms has rarely been reported.

In several feasibility studies, effective microorganism removal from stormwater has been shown, with *E. coli* and faecal coliforms removal in excess of 90% (Rusciano and Obropta, 2007; Hathaway et al., 2009; Chandasena et al., 2011). Even in non-vegetated biofilters, *E. coli* removal has been shown to be over 90% with the majority of bacteria being entrapped in the top layer of the filter media (Zhang et al., 2011). Thus, the filter media is the most important biofilter engineered component regarding microorganism removal. Furthermore, plants in biofilters can interfere with microbial removal performance by facilitating growth of beneficial bacteria, adsorption and predation of pathogens in the region of the soil under the immediate influence of living roots, termed rhizosphere (Bitton and Marshall, 1980; Rusciano and Obropta, 2007). In addition, plant root growth and die-off can help maintain hydraulic conductivity (Hatt et al., 2009), whereas the decayed roots may produce macropores or channelling in the media resulting in reduced filtration capacity of the filter media.

Stormwater events are highly variable in frequency, exposing biofilters to intermittent wetting and drying conditions. Drying might affect the structure and function of biofiltration system, e.g. fine fissures and macropores formation in filter media, preferential flow along dying roots, decreased plant activity, decreased biofilm activity (Blecken et al., 2009a). In addition, the level of moisture content in filter media can influence the survival of trapped microbes within the media (Schijven and Hassanizadeh, 2000; Stevik et al., 2004). However, the effect of this variability on microbial treatment by biofilters has regrettably been ignored. In addition, since viruses and protozoan differ from bacteria in survival, surface properties and size, their fate and transport in biofilters is expected to differ from that of bacteria. Hence, feasibility assessment and optimisation of removing viruses and protozoan by biofilters is a necessity.

This paper presents a laboratory study of biofilters at removing protozoan, bacterial, and viral indicators under intermittent wetting and drying conditions. The impact of several key biofilter design parameters including vegetation, filter media type, filter media depth and a saturated zone was investigated. The aims of the work were:

- To assess protozoan (*C. perfringens* spores), bacterial (*E. coli*), and viral (F-RNA coliphages) indicators removal by biofilters under the provision of regular stormwater inflow.
- To determine the influence of extended drying upon three indicators removal during subsequent storm events.
- To determine the optimal biofilter design for removal of three indicator organisms.

2. Materials and methods

2.1. Experimental set-up

28 biofilter columns were constructed from 375 mm diameter PVC pipes, with a transparent Perspex top section allowing for plant growth and ponding of water (Fig. 2). The inner walls of the filters were sand blasted in order to minimise preferential flow effects. Mesocosms were tested in a specially constructed greenhouse with a clear impermeable roof admitting full, natural sunlight.

To assess the influence of design parameters on indicator organisms removal, we tested six biofilter configurations using alterations of current biofilter design parameters (Table 1). ‘Standard’ biofilters consisted of 700 mm deep sandy loam (with a d_{50} of 0.25 mm) planted with *Carex appressa* which is commonly used in biofilters since it is instrumental for good removal of nutrients (Bratieres et al., 2008; FAWB, 2009). Sandy loam was used as the primary filter media for all configurations with basic characteristics shown in Table 2. ‘Non-vegetated’ biofilters (‘Unveg’) and ‘Short’ biofilters were created to determine the influence of vegetation and filter media depth on indicator organisms removal respectively. The same sandy loam media, mixed with 20% vermiculite and perlite (‘SLVP’ biofilters), was also trialled in this study, since it was shown to improve the removal of other pollutants related to human health risks (especially heavy metal removal because of the