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Reduction of bacteria and somatic coliphages in constructed wetlands for the treatment of combined sewer overflow (retention soil filters)

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ABSTRACT

Combined sewer overflows (CSOs) introduce numerous pathogens from fecal contamination, such as bacteria and viruses, into surface waters, thus endangering human health. In Germany, retention soil filters (RSFs) treat CSOs at sensitive discharge points and can contribute to reducing these hygienically relevant microorganisms. In this study, we evaluated the extent of how dry period, series connection and filter layer thickness influence the reduction efficiency of RSFs for *Escherichia coli (E. coli)*, intestinal enterococci (I. E.) and somatic coliphages. To accomplish this, we had four pilot scale RSFs built on a test field at the wastewater treatment plant Aachen-Soers. While two filters were replicates, the other two filters were installed in a series connection. Moreover, one filter had a thinner filtration layer than the other three.

Between April 2015 and December 2016, the RSFs were loaded in 37 trials with pre-conditioned CSO after dry periods ranging from 4 to 40 days. During 17 trials, samples for microbial analysis were taken and analyzed. The series connection of two filters showed that the removal increases when two systems with a filter layer of the same height are operated in series. Since the microorganisms are exposed twice to the environmental conditions on the filter surface and in the upper filter layers, there is a greater chance for abiotic adsorption increase. The same effect could be shown when filters with different depths were compared: the removal efficiency increases as filter thickness increases. This study provides new evidence that regardless of seasonal effects and dry period, RSFs can improve hygienic situation significantly.

1. Introduction

Combined sewer overflows (CSOs) emit various groups of pollutants into surface water bodies, including pathogens from wastewater. In Germany, vertical flow constructed wetlands (VF CWs, also known as retention soil filters (RSFs)) treat CSOs at sensitive discharge points. A primary stage constructed as sedimentation tank serves as trap for settleable particles to prevent clogging of the filter layer from overloading (Uhl and Dittmer, 2005). Other than intermittently charged VF CWs, CWs for the treatment of CSOs, so-called RSFs have long periods without feeding between such flooding events. Over time, secondary layers form on top of the filter material due to the surface filtration process and contain mostly organic material. These secondary filter layers themselves contribute to the overall sorption capacity of the filter (Pálfy et al., 2017).

Municipal wastewater from combined sewer systems contains a variety of pathogenic microorganisms such as bacteria and viruses that endanger human health, depending on their type and quantity. The discharged microbial load per event depends strongly on the catchment area, the season and the ratio of wastewater and stormwater. RSFs can contribute to reducing these hygienically relevant microorganisms.

RSFs reduce microorganisms with a combination of physical, chemical and biological processes. During a loading event, large amounts of combined wastewater are filtered over a short period of time. Solids are retained by filtration on the surface of the filter, whereas dissolved substances and very fine particles pass with the filtrate into the interior of the filter body and are retained by adsorption. Metabolism takes place in the dry period following the loading event when the filter body

Abbreviations: CSO, combined sewer overflow; RSF, retention soil filter; *E. coli*, *Escherichia coli*; EPS, extra polymere substances; I.E., intestinal enterococci; (VF) CW, (vertical flow) constructed wetland; WWTP, wastewater treatment plant; IBC, intermediate bulk container; MUD, 4-methylumbelliferyl-β-D-glucoside; MPN, most probable number; MSA, modified Scholtens agar; PFU, plaque forming units

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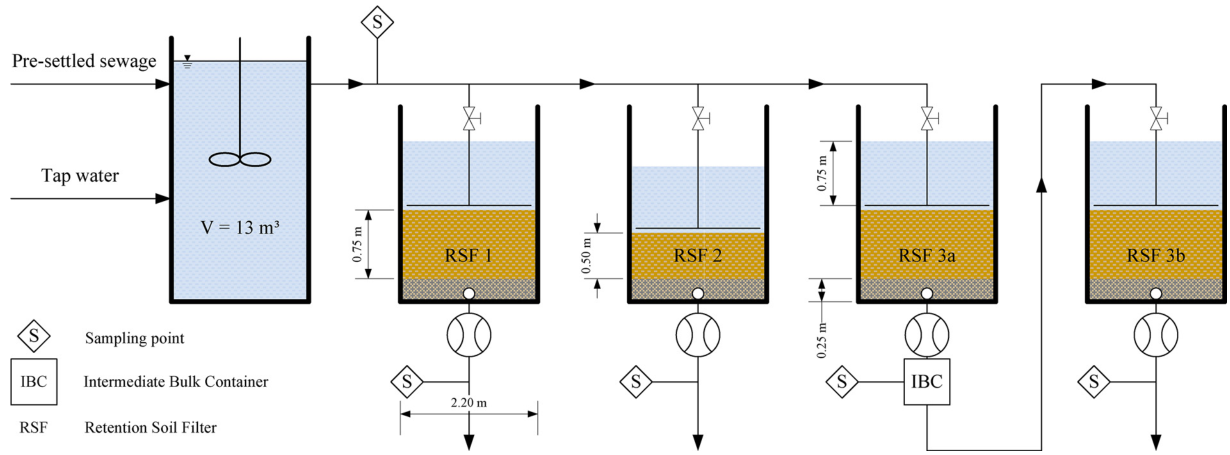

Fig. 1. Cross section of the pilot scale system.

is reaerated. Therefore, dry periods after the loading event are essential for undisturbed filter operation (Uhl and Dittmer, 2005).

In addition to environmental conditions such as pH, temperature, dissolved oxygen concentration and moisture content, competition and lack of nutrition not only play an important role in the reduction of bacteria, but also affect the growth and survival of microorganisms (Waldhoff, 2008).

The retention and elimination of pathogenic microorganisms, thus, is not isolated, but happens in parallel and simultaneously. Essential processes are filtration, adsorption and protozoan predation (Waldhoff, 2008).

Filtration can be divided into the effects of straining and surface filtration. Surface filtration applies to all particles that cannot pass the surface; it accounts as well for pathogens attached to particles or enclosed in particle flocks. Thus, most microorganisms attached to settleable solids will be filtered out to the greatest extent at the surface of the filter. In addition, since particles with a size > 5 µm can already be filtered out with the chosen type of filter material (Decamp et al., 1999; Weber-Shirk and Dick, 1997), some bacteria and protozoa in free phase will be retained on the filter surface. Moreover, viruses, bacteria and protozoa are present as bio-colloids. Due to straining, larger bio-colloids can be partially retained, but if the colloids are smaller than the pore diameter of the soil, the removal remains incomplete (Sirivithayapakorn and Keller, 2003).

In abiotic adsorption microorganisms become fixed to particles with electrostatic attraction between soil particles and bio-colloids with microorganisms, whereas in biotic adsorption microorganisms become attached to the substrate of a matrix of slimy extracellular polymeric substances in the biofilm (Waldhoff, 2008). However, abiotic adsorption is not considered as the dominant retention process for microorganisms in CWs for wastewater treatment. Rather, biodegradation through microorganisms, e.g. protozoa, eliminates pathogens following to biotic adsorption in biofilms (Stott and Tanner, 2005). The biofilm is built up on soil grains and plant roots and retains a variety of microorganisms. As phages or viruses are adhesive particles (Furiga et al., 2011, Gassilloud and Gantzer 2005), they bind to surfaces depending on their specific adsorption capacity (Zuang and Jin, 2003), in this case, especially on the sand grains of the filter body, but also on the organic material stored over time. Besides filtration and adsorption, protozoan predation is considered to be the most important elimination process of microorganisms (Stott et al., 2003). These unicellular organisms use bacteria and viruses as a nutrition source (Huws et al., 2005, Rønn et al., 2002).

In a previous study (Tondera et al., 2013), we evaluated if the reduction of the indicator organisms *Escherichia coli* (*E. coli*), I. E. and somatic coliphages depends on external conditions in the wetlands. Microbiological analyses were conducted at eight events from the influent and effluent of a large scale RSF with a surface layer of approximately 2200 m^2. The mean reductions of bacteria were 1.1 log$_{10}$ for *E. coli* and I. E., and 0.6 log$_{10}$ for somatic coliphages. As the conditions on a field scale were highly variable and the number of sampled events was low, it was not possible to evaluate any correlations between removal efficiencies and external influences such as weather conditions, antecedent dry periods, inflow concentrations or total inflow loads.

Accordingly, we investigated how the reduction of microorganisms depended on external conditions, such as antecedent dry periods and filter depth (75 cm of filter depth compared to 50 cm) under controlled conditions. Additionally, following a suggestion of Waldhoff (2008), that any kind of CWs could be combined to achieve higher treatment effect on nutrient removal, we investigated how a serial connection can improve the RSF's efficiency to reduce bacteria.

2. Methods

Four pilot scale RSFs were built on a test field at the wastewater treatment plant (WWTP) Aachen-Soers. To make them independent of weather occurrences, we placed them in a foil greenhouse. Conditioned CSO was generated in a storage tank of 13 m^3; wastewater from the outlet of the primary settler of the WWTP was mixed with non-chlorinated drinking water and rainwater at a ratio of 1–4.

The wetlands had a surface of approximately 4 m^2 each and a retention volume of 4 m^3. A cross-section of the set-up is shown in Fig. 1.

The filter material was sand (0/2 mm) with a steep sieve curve and 20 % of broken lime stone to prevent acidification, as suggested by the state guideline for the construction of RSFs (MKULNV, 2015). According to this guideline, the filter layers in RSF 1, 3a and 3b were 0.75 m high. While RSF 1 and 3a were replicates, RSF 3b was only loaded with the outflow of RSF 3a, which was stored in a buffer tank and discharged onto the surface of RSF 3b as soon as the buffer capacity of 300 L was reached. The process was repeated until RSF 3a was completely drained. RSF 2 had a filter layer of 0.5 m in order to investigate whether a smaller filter layer can reduce pathogen concentration sufficiently. The outflow of the RSFs was controlled with adjustable valves to approximately 0.03 L m^{-2} s^{-1} and started only after the retention volume reached a level of 0.75 m on top of the filter level. Completely draining each RSF took approximately 12 h.

2.1. Loading regime and sampling

Between April 2015 and December 2016, in 37 trials the RSFs were loaded after varying dry periods of 4–40 days. The conditioned wastewater – consisting of wastewater from the outlet of the primary settler of the WWTP and drinking water at a ratio of 1:4 – was stirred in the storage tank in order to create homogenized inflow conditions. Thus,