

Performance of retention soil filters for the reduction of hygienically-relevant microorganisms in combined sewage overflow and treated wastewater

N. Zacharias, S. M. Essert, A. F. Brunsch, E. Christoffels, T. Kistemann and C. Schreiber

ABSTRACT

Environmental quality standards for surface waters have been significantly expanded through recent amendments to German regulations. Limit values are only established for applicable regulations if the water is indicated for certain uses, e.g. abstraction of irrigation water. Nevertheless, surface water bodies are often used for hygiene-sensitive purposes. In the course of climate change, stronger precipitation events will occur, which may lead to more frequent loading and discharge of combined sewer overflow (CSO) into surface water bodies. Retention soil filters (RSFs) are attracting attention as an extensive treatment technology for CSO and additional wastewater treatment. This study examined large-scale RSFs for CSO treatment, as well as the effectiveness of RSFs as a fourth purification stage. An RSF test facility was established at a municipal wastewater treatment plant (WWTP), consisting three semi-technical RSFs that were fed exclusively with treated water from the WWTP. The reduction of microorganisms mostly occurred within the first centimeters of the RSFs. For most hygienic-microbiological parameters, a 1–2 log unit reduction could be detected in addition to the reduction within the WWTP. Antibiotic-resistant bacteria were reduced to the same extent. Investigation of the large-scale RSFs showed that a flow rate reduced by half corresponded to better reduction performances.

Key words | antibiotic-resistant bacteria, bioretention filter, constructed wetlands, fourth purification stage, human-pathogenic microorganisms, sewage treatment plant

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INTRODUCTION

Retention soil filters for treatment of combined sewer overflow (CSO) and as a fourth purification step in wastewater treatment plants

In the course of climate change, precipitation patterns are expected to change in terms of quantity, incidence and frequency, which may lead to more frequent load and discharge of CSO and higher release from the unsealed

landscape. Increased microbial pollution from discharge events of CSO after heavy precipitation events could be shown in other studies (Gibson *et al.* 1998; Kistemann *et al.* 2004; Rechenburg & Kistemann 2009). In order to reduce the pollution of surface waters after CSO introduction, in recent years treatment of the discharged water with retention soil filters (RSFs), a special type of vertical constructed wetlands, has been utilized in Germany. RSFs

for CSO treatment are fed with mixed water of combined sewer systems and relieve the water loads out of overflow basins, which led to decreased pollution in case of heavy precipitation events. Thus, they can significantly reduce microorganisms and hydraulic loads within the receiving surface water (Scheurer *et al.* 2015). RSFs investigated as a fourth purification step in wastewater treatment plants (WWTPs) show high performance in reducing dissolved organic carbon and micropollutants (Brunsch *et al.* 2018).

Hygienically relevant microorganisms in wastewater

Hygienically relevant parameters in WWTP influents and effluents are well known. Common parameters for the microbiological characterization of wastewater include total and fecal coliform bacteria, fecal streptococci, clostridia, salmonellae, cryptosporidia, *Giardia* and enteropathogenic viruses (Rechenburg *et al.* 2006; Wingender 2011). Antibiotic-resistant bacteria (ARB) in surface waters can play a role in the risk of using these waters (Müller *et al.* 2018; Döhla *et al.* 2019). The actual health risk cannot be evaluated yet because there are not enough data on ARB in surface waters. Nevertheless, to protect people who come into contact with surface waters and to reduce ARB into the environment, the microbiological purification effectiveness of WWTPs may be enhanced as it was shown that important human-pathogenic ARB can reach the environment from WWTP discharge (Baquero *et al.* 2008; Novo *et al.* 2013). Scheurer *et al.* (2015) investigated an RSF, which was installed to relieve CSO. It could be shown that this RSF was very suitable to reduce the concentration of microorganisms (*Escherichia coli*, enterococci and staphylococci) by 2–3 log units and reduced the percentage of ARB. Thus, RSFs represent a great potential to purify treated wastewater from different hygienically relevant microorganisms. Despite promising studies (Mertens *et al.* 2012; Christoffels *et al.* 2014; Tøndera *et al.* 2019), RSFs have not yet been put into operation for extensive long-term monitoring. This study investigated the purification performance of semi-automatic RSFs on different facultative-pathogen microorganisms (including ARBs) as a fourth purification stage in the form of a pilot test facility.

MATERIALS AND METHODS

Large-scale RSFs

This study uses microbiological data, collected by sampling two different RSFs for CSO treatment. At both large-scale RSFs, 25 feeding events per RSF were investigated. Both RSFs are located in Germany and operated by the local water board (Erftverband). The filter height and material of the two RSFs is the same, with a height of 75 cm and a mixture of materials containing 3% clay, 17% fine sand, 59% medium sand, 20% large grained sand and 1% gravel. The sand is supplemented with CaCO₃ and is used as filter material to maintain a constant pH. The vegetation on top to prevent clogging is reed (*Phragmites australis*). The effective drainage area of RSF A is approximately 19 ha, with a filter size of 707 m² and a retention volume of 782 m³. The connected sewer system at RSF B drains the waste- and rainwater of a catchment area with approximately 7,700 inhabitants. The drainage effective area amounts to approximately 79 ha. The filter area is 1.495 m², and the retention volume is 2,630 m³. The catchment areas of both RSFs are characterized by rural residential areas with some agricultural enterprises. There are no significant commercial or industrial areas or buildings with special wastewater (such as hospitals). Both filters were put into operation in 2005. The filtered water is introduced into the receiving surface waters with a maximum hydraulic retention time (HRT) of 0.03 L/(s*m²) at both filters. The de facto HRT at RSF A could be determined as being only 0.015 L/(s*m²) during the sampling campaign.

RSF semi-technical test facility

In order to investigate the effectiveness of RSFs as an additional purification stage for sewage treatment plants, an RSF test facility was established at a municipal WWTP in Germany, as part of the project 'Transnational Action Programme On Emerging Substances' (TAPES 2016). Purification performance of this RSF facility concerning micropollutants and dissolved organic carbon was previously published (Brunsch *et al.* 2018). This facility consists of three semi-technical RSFs, each with a 1.5 m²

filter area and a volume of 1.4 m³. Two of those test facilities (filter 1 and filter 2) were investigated in this study. Both filters contain original material from large-scale RSF systems, which were already in operation for several years. The material of filter 1 corresponds to RSF A and filter 2 corresponds to RSF B (see above). The semi-automatic facilities filter 1 and filter 2 were fed exclusively with treated wastewater from the WWTP. One load lasted 28 h, followed by a 56-h dry phase. The filters were charged at 0.03 L/(s·m²) which meets the requirements of the German guidelines and standards for RSFs (MUNLV 2015; DWA 2019) and the filtration performance of large-scale installations. These feeding cycles ensured that aerobic conditions could be established in the filters during the dry and wet phases. Water filling level during feeding cycles was about 10–15 cm above the filter material surface. Total feeding volumes were approximately 1,300 m³ for filter 1 and filter 2 (Brunsch *et al.* 2018). In addition to the inflows and outflows, water from individual filter layers (0.1 m, 0.3 m, 0.75 m) were sampled by means of built-in sampling tubes. For each semi-technical filter, 14 feeding events were investigated.

Sampling

An automatic sampling system was installed to sample from the large-scale RSFs. The start of the water draining through the RSF was registered via a water level sensor in the inlet of the RSF, and the sampler of the inlet was activated. Effluent sampling was performed with a corresponding delay (40 min). This design ensured that the residual water from the last warding that was necessary for operation was not sampled. For microbiological analysis, submersible pumps filled 200-L sample containers (for influents and effluents) in approximately 15 min. Sampling of the semi-technical RSF facility was done randomly within the 28 h loading event. The pilot plant feed was taken from the WWTP effluent drainage pit. To sample at the respective depth and effluents, the sample was taken from the sampling tap of the respective RSF after the stagnation water had run out. The samples were transported refrigerated to the laboratory, and processing was usually started immediately (but no later than 24 h after sampling). Any necessary intermediate storage took place overnight at 5 ± 3 °C in the refrigerator.

Cultural detection of microorganisms and calculation of the reduction performance

Due to the high solid loads for the CSO and expected bacterial loads, samples were diluted with sterile 0.9% NaCl. For parameters with lower expected concentrations, samples added to agar plates via a membrane filter using a vacuum pump and membrane filtration unit (Whatman). Analysis of the individual microbiological parameters was performed according to normalized standard methods or based on published methods. The analyzed microorganisms shown in this study are *E. coli* (DIN EN ISO 9308-1:2012, modified by using an antibiotic supplement), somatic coliphages (DIN EN ISO 10705-2:2002), as an indicator for enteropathogenic viruses, and the parasite *Giardia lamblia* (ISO 15553:2006, EPA Method 1623, HMSO:1989). Additionally, ARB were investigated within the project 'HyReKA' (biological and hygienic-medical relevance and control of antibiotic-resistant pathogens in clinical, agricultural and municipal wastewater and their relevance in raw water), funded by the German Federal Ministry of Education and Research. The ARB parameter shown in this study is extended-spectrum beta-lactamase (ESBL)-producing *E. coli*; the utilized method was developed and adapted for the HyReKA project (Müller *et al.* 2018) and will soon be published in detail (Schreiber *et al.*, in preparation). The sample was placed on ChromESBL agar (Co. Mast) and incubated for 24 h at 42 °C.

The microorganisms concentrations were calculated in logarithmic units (log units) of colony-forming units (cfu) or plaque-forming units (pfu) per 100 mL for each microbiological parameter. The difference in log units between the effluent and influent from the RSFs or WWTP was used to measure the reduction performance.

RESULTS

Reduction performance of large-scale RSFs

Our data revealed that the load of all tested microbiological parameters was reduced by passage through the RSFs in both test scenarios (large-scale and test facility). Figure 1 shows the reduction performance of the two investigated

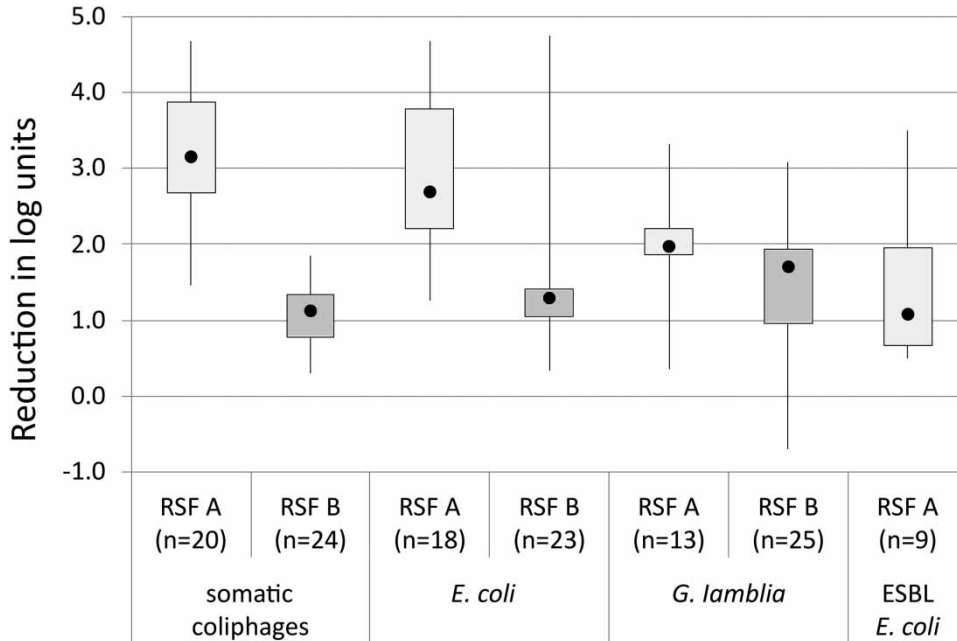


Figure 1 | Boxplots for the reduction performances of the large-scale filters (A and B) in log units for somatic coliphages, *Escherichia coli*, *Giardia lamblia* and ESBL-producing *E. coli*. The individual sample number (n) is based on the number of samples where exact concentration were obtained, categorical results (e.g. <10) were excluded in the displayed data.

large-scale RSFs. The median reduction performance at RSF A was up to 2 log units higher than at RSF B for all investigated parameters at both large-scale filters (not all data shown). For interpreting the results, it is important to notice that the concentrations of the combined sewage discharging into the large-scale RSFs were similar; the only difference was a slightly lower concentration (median) of the bacterial parameters at RSF B. The *E. coli* concentrations ranged from <1,000 to 5,450,000 cfu/100 mL (median 1,325,000 cfu/100 mL) at RSF A and 216,220 to 6,360,000 cfu/100 mL (median 620,000 cfu/100 mL) at RSF B. The somatic coliphage concentrations were <100 to 242,100 pfu/100 mL (median 41,800 pfu/100 mL) at RSF A and 8,000 to 269,000 pfu/100 mL (median 51,550 pfu/100 mL) for RSF B. ESBL-producing *E. coli* could only be investigated at one of the large-scale filters (RSF A); the detected concentrations were between 37,273 and 909,091 cfu/100 mL (median 263,636 cfu/100 mL). The concentrations of the parasitic parameters in the combined sewage were slightly higher at RSF B, from 61 to 28,000 cysts/100 L (median 1,200 cysts/100 L), compared to 38 to 10,303 cysts/100 L (median 400 cysts/100 L) at RSF A.

Reduction performance at the semi-technical facility

The reduction performance of the filters at the semi-technical facility for treated wastewater as a fourth purification stage reduced most microorganisms of 1–2 log units. Even some of the bacteria were below the corresponding detection limit. The results of the ‘HyReKA’ project indicate RSFs diminished ARB: there was a 2.26-log-unit (median) reduction (Table 1).

It was not possible to investigate the reduction performance for the parasitic parameters at the test facility because of the relatively small 0.5–1 L sample sizes, whereas the parasitic analysis needs sample volumes up to 100 L or more, especially if concentrations are relatively low as in the case of treated wastewater. Table 1 shows the individual reduction performances as measured at the large-scale RSFs compared to the corresponding semi-technical filters. The reduction performances for *E. coli* and somatic coliphages differed between RSF A and B in large-scale applications (see also Figure 1), where RSF A exhibited greater reduction (despite using the filter material from the large-scale filters). The measured concentrations cannot be directly compared

Table 1 | Reduction performances (in log units) of the large-scale filters (A and B) and the corresponding semi-technical test facilities (1 and 2)

			Reduction in log units			
			RSF A	Test facility (1)	RSF B	Test facility (2)
Somatic coliphages	[pfu/100 mL]	<i>N</i>	24	14	24	14
		<i>Min</i>	1.47	1.08	0.31	1.08
		<i>Median</i>	3.15	2.46	1.12	2.53
		<i>Max</i>	4.68	3.3	1.84	3.3
<i>E. coli</i>	[cfu/100 mL]	<i>N</i>	25	6	23	6
		<i>Min</i>	1.27	1.25	0.64	0.92
		<i>Median</i>	2.66	2.02	1.24	1.76
		<i>Max</i>	4.7	3.81	4.95	3.33
ESBL- <i>E. coli</i>	[cfu/100 mL]	<i>N</i>	9	5	–	5
		<i>Min</i>	0.50	1.26	–	–0.07
		<i>Median</i>	1.08	2.26	–	2.26
		<i>Max</i>	3.50	3.69	–	3.69

RSF A corresponds to the material from filter 1 and RSF B to filter 2.

due to the different influent matrices, but the reductions are calculated within each system and are thus deemed to be comparable.

Individual filter layer

The semi-technical test facility was constructed to sample at different filter depths and thus to investigate the reduction performance at the different material steps. The microorganism reduction performance differed depending on the layer depth (Figure 2). In most cases, the strongest reduction occurred at 0.1–0.3 m.

DISCUSSION

RSF reduction performances

CSO consists of sewage as well as the run-off water from sealed surfaces. Microbial concentrations in CSO may be affected by the so-called first flush of stormwater runoff in the form of solid and sediment remobilization (Gupta & Saul 1996; Barco *et al.* 2008). The measured concentrations of microorganisms in the inflow of the investigated RSFs were similar, data that demonstrate the influent concentrations were not responsible for the reduction performance differences. This study emphasizes RSFs for their

hygienic-microbiological purification performance of human-pathogenic bacteria and parasites in combined sewage or purified wastewater. The reduction performances of the investigated RSFs are in line with reduction rates of indicator bacteria in conventional RSFs previously reported. Median reductions reported range from up to 3 log units (Merkel & Schaule 2010; Christoffels *et al.* 2014; Schreiber *et al.* 2016) to lower than 2 log units (Orb 2012; Tondera *et al.* 2013).

Results from semi-technical RSF facilities already show strong purification performance for chemical substances (Brunsch *et al.* 2018). Investigation into the removal of human-pathogenic bacteria and ARB (*E. coli*, enterococci and staphylococci) in CSO by a full-scale RSF was reported by Scheurer *et al.* (2015). Their measured RSF reduction performance of these bacteria ranged from 2 to 3 log units. This phenomenon was confirmed by RSF A; it diminished ESBL-producing *E. coli* 1.08 log units, *E. coli* 2.66 log units and somatic coliphages 3.15 log units (median values). Ruppelt *et al.* (2018) reported similar reductions on pilot scale systems between 1 and 2 log units for faecal indicator bacteria and between 1.0 and 1.2 log units for somatic coliphages; straining processes and adsorption serving as the main removal mechanisms. Reduction rates could be further improved by using a series connection of two RSFs with a filtration layer of 0.75 m each. In this study, the large-scale RSF A demonstrated all in all better results in

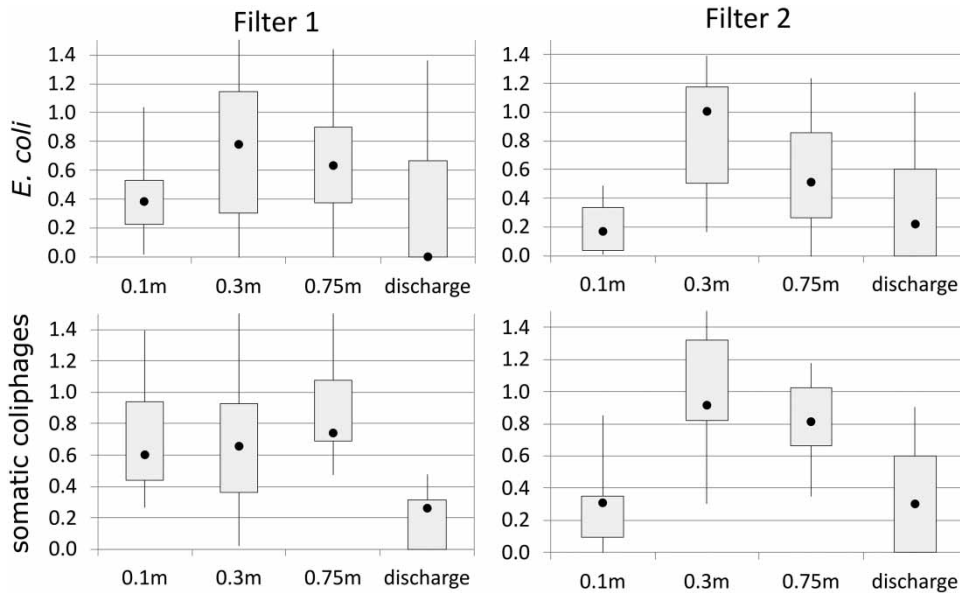


Figure 2 | Reduction of somatic coliphages ($n = 14$) and *E. coli* ($n = 14$) concentrations by RSFs A and filter B for treated wastewater. The layer depth (0.1, 0.3 and 0.75 m) or the discharge is shown on the x-axis. The y-axis indicates the reduction (in log units) of *E. coli* and somatic coliphages.

reduction performance. Considering the fact that we compared very different ranges of input concentrations between the large-scale RSFs (input was combined sewage) and the semi-automatic test filters (input was treated wastewater), RSF A and B performance easily indicates multiple log-step reductions, a finding that suggests a log level ratio with higher treatment efficiency than the test facilities. Nevertheless, it must be noted that the filter material is not responsible for the different reduction performance in the large-scale study, because the material does not differ in the investigated RSFs.

In comparison to UV disinfection, activated carbon filtration/adsorption, ozonation and membrane bioreactors, RSFs offer similar or even better reduction performances as a fourth purification stage. Disinfection reduced non-resistant bacteria during chlorination by 3 log units in the study of Huang *et al.* (2011). Czekański *et al.* (2016) confirmed ARB removal of 1–2 log units using ozonation. Brunsch *et al.* (2018) described that RSFs are effective in removing several organic micropollutants from WWTP effluent, and that the organic matter content that increases by operation time enhances organic micropollutant removal. Thus, an RSF is a natural system and needs a longer start up period until operating at full capacity compared to technical

treatment systems such as activated carbon filtration or ozonation. Nevertheless, chlorination or ozonation can remove organic trace elements and are dose-dependent in their limited effect on eliminating microorganisms (Rudolph *et al.* 1993). This study showed substantial reductions in somatic coliphages (median 2.46/2.53 log units for filter 1/filter 2), *E. coli* (median 2.02/1.76 log units) and ESBL-producing *E. coli* (median 2.26/2.26 log units) in treated wastewater at the test facilities. Classical treatment plants offer a median bacterial reduction of 2 log units (range of 1.5 to 3) and 1 to 3.6 log units for parasites (SWIST I 2001). Dizer *et al.* (1994) report reduction values with micro-filtration from 1 to >6 log units for bacteria, 1–6 log units for virus and >4 log units for parasites. According to Fiksdal & Leiknes (2006), ultrafiltration as a treatment technology diminishes all parameters (bacteria, viruses and parasites) by >4 log units. However, it does not need extensive maintenance, unlike the other discussed methods: ultrafiltration requires filter backwashes, active charcoal must be reactivated, and for ozonation, the formation of non-specific by-products needs to be removed along with any residual ozone. Additionally, RSFs are efficient in terms of costs (materials, maintenance and repairs). Thus, considering the purification performance, RSFs should be noticed for

their potential option as a fourth stage for wastewater treatment.

Individual filter layer

Waldhoff (2008) states that, on average, approximately 40% of bacteria are reduced in the top 10 cm of an RSF. According to Orb (2012), the value is even over 50%. Over time, an accumulated organic material layer also forms at this depth, and this material influences the reduction performance. This secondary filter layer of approximately 2 cm is formed at the sand–water interface by suspended solids in the water and dead plant tissue. The subsequent upper, biologically active filter layer (3–5 cm) develops depending on filter granulation and speed (Orb 2012). A presumed mechanism that underlies this effect could increase adsorption due to biofilm growth (Waldhoff 2008). Orb (2012) explains the high microbiological retention in the secondary filter layer and upper, biologically active layer by lower sieving of the pore channels and the high organic content (approximately 90%), both of which increase bacterial adhesion. In contrast, the current data revealed that the highest reduction occurred in the layer between 0.1 and 0.3 m, a top layer of the filter with a high concentration of accumulated biofilm and organic material. Thus, this layer may promote the microorganism and trace element reductions.

Flow rate

RSF reduction performance varies depending on their operation. The efficiency in bacterial reduction was different between the two large-scale RSFs, which provided the filter material for the semi-technical filters. Reduction performance was much higher in RSF A, which had an effective HRT of $0.015 \text{ L}/(\text{s}\cdot\text{m}^2)$ compared to RSF B at $0.03 \text{ L}/(\text{s}\cdot\text{m}^2)$. Both of these values conform to the German guidelines on RSF construction. Influences like filter structure and influent composition can be eliminated as reason for the disparate reduction performances by examining the individual CSO compositions in the large-scale experiments and the use of the same operation conditions in the semi-technical filters. The latter two operated at the same flow rate ($0.03 \text{ L}/(\text{s}\cdot\text{m}^2)$) and did not show any significant differences in their purification performance. Thus, the

rate of throttle drainage, based on the structural parameters discussed herein, appears to be the deciding factor in the effectiveness of the RSF's hygienic-microbiological reduction performance.

CONCLUSIONS

The investigated semi-technical RSF facilities offered high efficiency in reducing the concentration of microorganisms that comprise ARB. Additionally, an influence of the HRT on the reduction performances was revealed, because the higher reduction rate as detected at the large-scale RSF A could not be confirmed for the semi-technical filter 1: the reduction performance was therefore determined for the large-scale filters as being inversely proportional to the flow rate. Thus, this factor is crucial for RSF purification performance concerning health-relevant microorganisms. Additionally, the individual depths of the filters offered different purification performance, which was highest within the upper layer (0.1–0.3 m depth). Overall, RSFs are very suitable as a fourth purification stage, because they efficiently reduce hygienic-microbial pollution such as parasites, viruses and bacteria including ARB. The bacterial reduction performance was at the same or even better value than UV disinfection, activated carbon filtration/adsorption, ozonation and membrane bioreactors, all of which are the standard fourth purification stage in WWTPs. Considering RSFs as a fourth purification step in WWTPs, they should be further investigated to increase their efficiency in the reduction of hygiene-relevant microbial pollution.

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