Ecological Engineering 95 (2016) 635–644

Contents lists available at ScienceDirect

Ecological Engineering

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

Removal of pathogen indicators from secondary effluent using slow sand filtration: Optimization approaches

Eva M. Seeger, Mareike Braeckevelt*, Nils Reiche, Jochen A. Müller, Matthias Kästner

Department of Environmental Biotechnology, Helmholtz Centre for Environmental Research – UFZ, Permoserstrasse 15, 04318 Leipzig, Germany

ARTICLE INFO

Article history:
Received 2 September 2015
Received in revised form 31 May 2016
Accepted 16 June 2016

Keywords:
Escherichia coli
Enterococci
Clostridium perfringens
Bacteriophages
Schmutzdecke
Wastewater reuse

ABSTRACT

In many arid regions, the reuse of wastewater is an economic option for crop irrigation. To avoid health risks for consumers, pathogens must be eliminated prior to application. Slow sand filtration (SSF) represents an effective low-tech treatment technology for pathogen removal from water. To further improve the time-space yield of SSF, innovative filter configurations were investigated regarding the removal of the pathogen indicators *Escherichia coli*, enterococci, *Clostridium perfringens* spores, somatic and F-specific RNA coliphages as well as heterotrophic bacteria. A standard filter (***N***), a recirculating filter (***R***), a static cascade (***N+N***) and a rotating cascade (***C***) were tested at high and low hydraulic loading rates, two recirculation rates and two rotation frequencies. Results showed that only ***C*** and ***N+N*** concurrently complied with European standards for *E. coli* and enterococci, achieving mean log removal of 2.7-4.7 and 2.1-2.4, respectively. The best performance was reached by ***C*** with weekly rotation; ***N+N*** may be a promising, technically simpler alternative. The crucial role of biological removal mechanisms for *E. coli* and enterococci elimination was indicated by (i) the increased efficiency of the standard SSF ***N*** after 1½ years of operation and (ii) the positive impact of several *Schmutzdecke* layers. *C. perfringens* spore removal performance was good for all SSFs. Considerable sorption of spores was indicated by decreased efficiency in ***N*** and ***C*** at long operation times. Somatic coliphages were reduced to concentrations close to the detection limit, while F-specific RNA coliphage removal was ~1.1 log. Removal of heterotrophic bacteria was generally limited.

1. Introduction

In many arid regions of the world, the demand for irrigation water in agriculture periodically or permanently exceeds the available water resources (Pedrero et al., 2011). Water scarcity is aggravated by population growth, intensified agricultural practices, deterioration of soils and climate change leading to less precipitation (Rosegrant et al., 2009).

To mitigate this problem, the use of wastewater for irrigation is more and more widely applied with the added benefit of utilizing its considerable nutrient content (Pedrero et al., 2011; Norton-Brandão et al., 2013). The use of insufficiently treated or untreated wastewater is problematic, partly due to undesirable levels of salinity and heavy metals (Norton-Brandão et al., 2013) but mainly due to its pathogen load stemming from human and animal excreta. Pathogens may contaminate crops and pose health risks for agricultural workers, crop handlers and consumers (Schaefer et al., 2004). Thus, wastewater should be treated for pathogen removal in addition to primary treatment for removal of Chemical and Biological Oxygen Demand (COD/BOD), salt and metals, prior to reuse in agriculture. For unrestricted irrigation of human food crops intended for raw consumption, all relevant guidelines and regulations require coliform indicators (total/faecal/thermotolerant coliforms) to be below 10^3 Colony Forming Units (CFU)/100 ml (WHO 1989; ANZECC, 2000; US EPA 2004), while stricter European

Abbreviations: BOD, Biological Oxygen Demand; CFU, Colony forming units; COD, Chemical Oxygen Demand; CWs, Constructed wetlands; DOC, Dissolved Organic Carbon; FC, faecal coliforms; HLR, hydraulic loading rate; HPC, Heterotrophic Plate Counts; HSSF, horizontal subsurface flow; MPN, Most Probable Number; PFU, Plaque Forming Units; PVC, polyvinyl chloride; R2A agar, reasonerís 2A agar; SCE, secondary clarifier effluent; SPS agar, sulfite polymyxin sulphadiazine agar; SSF, slow sand filtration/filter; TC, total coliforms; TN, total nitrogen; TOC, total organic carbon; WWTP, wastewater treatment plant.

* Corresponding author. Present address: Center for Advanced Water Research, Faculty of Environmental Sciences, Technical University of Dresden, Bergstraße 66, 01062 Dresden, Germany.
E-mail address: mareike.ursula.braeckevelt@tu-dresden.de (M. Braeckevelt).

http://dx.doi.org/10.1016/j.ecoleng.2016.06.068
0925-8574/

Table 1
Biological characteristics of the secondary clarifier effluent (SCE) used as the inflow water for the Slow Sand Filter (SSF) systems and of the outflow from the horizontal subsurface flow constructed wetlands (HSSF CWs) at the Langenreichenbach wastewater treatment facility.

Parameters	Units	Secondary clarifier effluent			HSSF CW effluent		
		Mean ± σ	Median	N	Mean ± σ	Median	N
Total Coliforms	CFU/100 ml	$5.8 \times 10^4 \pm 5.4 \times 10^4$	4.1×10^4	25	–	–	–
E. coli	CFU/100 ml	$8.9 \times 10^3 \pm 8.2 \times 10^3$	7.0×10^3	41	$3.0 \times 10^5 \pm 1.9 \times 10^5$[b]	–	42
Enterococci	CFU/100 ml	$2.1 \times 10^3 \pm 2.0 \times 10^3$	1.4×10^3	42	$2.0 \times 10^4 \pm 1.5 \times 10^4$	1.9×10^4	5
Heterotrophic bacteria	CFU/100 ml	$1.2 \times 10^6 \pm 2.1 \times 10^6$	5.5×10^5	39	$1.1 \times 10^7 \pm 1.5 \times 10^7$	4.3×10^6	3
Presumptive *Clostridium perfringens* spores[a]	CFU/100 ml	$8.1 \times 10^2 \pm 5.7 \times 10^2$	7.0×10^2	33	$1.0 \times 10^5 \pm 2.0 \times 10^5$	3.2×10^3	4
F-specific RNA coliphages	PFU/100 ml	$6.4 \times 10^1 \pm 2.8 \times 10^1$	4.9×10^1	3	–	–	–
Somatic coliphages	PFU/100 ml	$3.5 \times 10^3 \pm 3.1 \times 10^3$	2.2×10^3	14	$1.6 \times 10^5 \pm 1.4 \times 10^5$	1.1×10^5	5

[a] Spores of anaerobic Clostridia grown on SPS medium.
[b] Headley et al. (2013).

guidelines call for *E. coli* loads below 200 or 100 CFU/100 ml (DIN, 1999; Italian Decree, 2003; Spanish Royal Decree, 2007).

Constructed wetlands (CWs) represent a low-tech and effective treatment technology for removal of pathogens. However, reported effluent loads of total coliforms (TC), faecal coliforms (FC) and *E. coli* from secondary subsurface flow CWs (TC: 4×10^6, FC: 9×10^5, *E. coli*: 3×10^3–2×10^6 CFU/100 ml) and free water surface (FWS) CWs (TC: 3×10^5–4×10^6, FC: 2×10^4–4×10^4, *E. coli*: 2×10^6 CFU/100 ml) (Decamp and Warren, 2000; Masi et al., 2004; Vymazal, 2005; Ghermandi et al., 2007; García et al., 2013; Headley et al., 2013; Abou-Elela et al., 2014; Wu et al., 2016) are too high for a safe reuse even in restricted irrigation (US EPA, 2004; WHO, 2006; Ghermandi et al., 2007; Norton-Brandão et al., 2013). In order to provide irrigation water quality, CW effluents require further disinfection steps. It has been suggested to apply ozone (Miranda et al., 2014) or UV (Toscano et al., 2013) for CW effluent disinfection, but simple and low-cost post-treatment options would be preferable.

Continuous Slow Sand Filtration (SSF) is a low-tech process that has been used for pathogen and particle removal in drinking water purification for decades (Logsdon et al., 2002), and can also be implemented under restricted conditions with locally available materials. Water continuously percolates through a sand column utilizing the pressure of a permanent water head. On the filter surface, a biologically active compartment (*Schmutzdecke*, German for 'dirt layer') forms where most of the pathogen removal takes place (Langenbach et al., 2009; Pfannes et al., 2015). Pathogen retention is mainly due to straining and adsorption (Stevik et al., 2004), while pathogen inactivation is caused by abiotic and biotic mechanisms. Natural die-off such as starvation, predation by eukaryotic bacterivores (protozoa and heterotrophic nano-flagellates) and bacteria such as *Bdellovibrio* sp., as well as lysis induced by bacteriophages and algal-derived reactive oxygen species have been identified as contributing factors (Weber-Shirk and Dick, 1997, 1999; Stevik et al., 2004; Wand et al., 2007; Haig et al., 2015).

In recent years, continuous SSF has gained increasing attention as a promising technology for disinfection of secondary effluent with the purpose of reuse (Adin, 2003; Christou et al., 2014). A wide range of removal efficiencies for TC (0.3–3.5 log units), FC (2-2.4 log-units), *E. coli* (1.9–4.1 log units) and enterococci (0.7-3.7) has been reported (Ellis, 1987; Farooq and Alyousef, 1993; Sadiq et al., 2003; Mälzer, 2005; Keraita et al., 2008; Langenbach et al., 2009, 2010; Bauer et al., 2011; Kader Yettefti et al., 2013), using various sand materials, filter designs and hydraulic loading rates (HLRs). Intermittent SSF, also known as Infiltration Percolation, is characterized by lower HLRs and pathogen indicator removal efficiencies (Young-Rojanschi and Madramootoo, 2014). Research conducted on recirculating SSF focused on intermittently loaded systems with recirculation tanks as specified by US EPA (1980), dealing with primary effluent or high strength wastewater (Gold et al., 1992; Healy et al., 2007). The use of SSFs with direct recirculation for tertiary treatment has not yet been reported.

Regarding continuous SSF, systematic approaches to optimize sand grain size distribution and operation mode (HLR, hydraulic head) for pathogen removal are scarce (Langenbach et al., 2009; Bauer et al., 2011; Kader Yettefti et al., 2013). Sand with an effective grain size d_{10} between 0.15 mm and 0.4 mm and a uniformity coefficient of $U < 5$ is recommended for drinking water purification with HLRs of 5–40 cm h^{-1} (Sánchez et al., 2006). Langenbach et al. (2009, 2010) have investigated removal of *E. coli* and enterococci using various sand grain size distributions and HLRs. Results indicate that a fine and uniform sand material with a high sand surface area achieves the best faecal indicator removal at HLRs of 5 and 10 cm h^{-1}.

For investigations on post-treatment of CW effluents, it is appropriate to consider horizontal subsurface flow (HSSF) CWs as the simplest technology (free water surface CWs were ruled out due to potential public health issues such as mosquitos). Taking into account reported secondary subsurface flow CW effluent qualities and SSF removal efficiencies for *E. coli*, post-treatment of CW effluent in SSFs can potentially produce *E. coli* outflow concentrations between zero and 10^4 CFU/100 ml. Thus, although optimally performing system combinations may be able to reduce *E. coli* loads to values falling below the limits defined in European irrigation water standards, many CW-SSF system combinations will not reach the specified goals (DIN, 1999; Italian Decree, 2003; Spanish Royal Decree, 2007). For the potential employment of SSF in hybrid CW-SSF units treating wastewater for safe reuse in irrigation, a further SSF performance optimization is needed in order to guarantee the compliance of effluents with established limits.

Thus, the goal of the present study was to compare the pathogen indicator removal efficiency of four different SSF designs: standard, recirculating, a static series of two SSFs, and a rotating cascade. The underlying aim was to improve SSF performance by an enhanced use of the biologically active *Schmutzdecke* layer(s) by (i) increasing the contact time between the wastewater and the active layer via partial recirculation of the effluent, or by (ii) formation of several active layers when operating multiple SSFs either in a static series or as a rotating cascade. By periodically rotating the wastewater recipient order in a SSF cascade, the formation of several *Schmutzdecke* layers could be promoted, each receiving high loads of organic carbon, nutrients and microbial matter in turn. So far, investigations on SSF cascades have very rarely been reported (e.g. Kadewa et al., 2010); to the best of the authors' knowledge, no results have been published concerning pathogen removal in (rotating) SSF cascades.

Removal of indicators for wastewater pathogen groups relevant for irrigation water quality was investigated: aerobic bacterial load (Heterotrophic Plate Counts, HPC) and faecal bacterial indicators (*E. coli* and the more stress-resistant enterococci); pathogenic protozoa forming (oo)cysts such as *Cryptosporidium parvum* and *Giardia lamblia* (represented by *Clostridium perfringens* spores with similar characteristics regarding treatment: long-term survival, high resistance to disinfection, removal predominantly by fil-

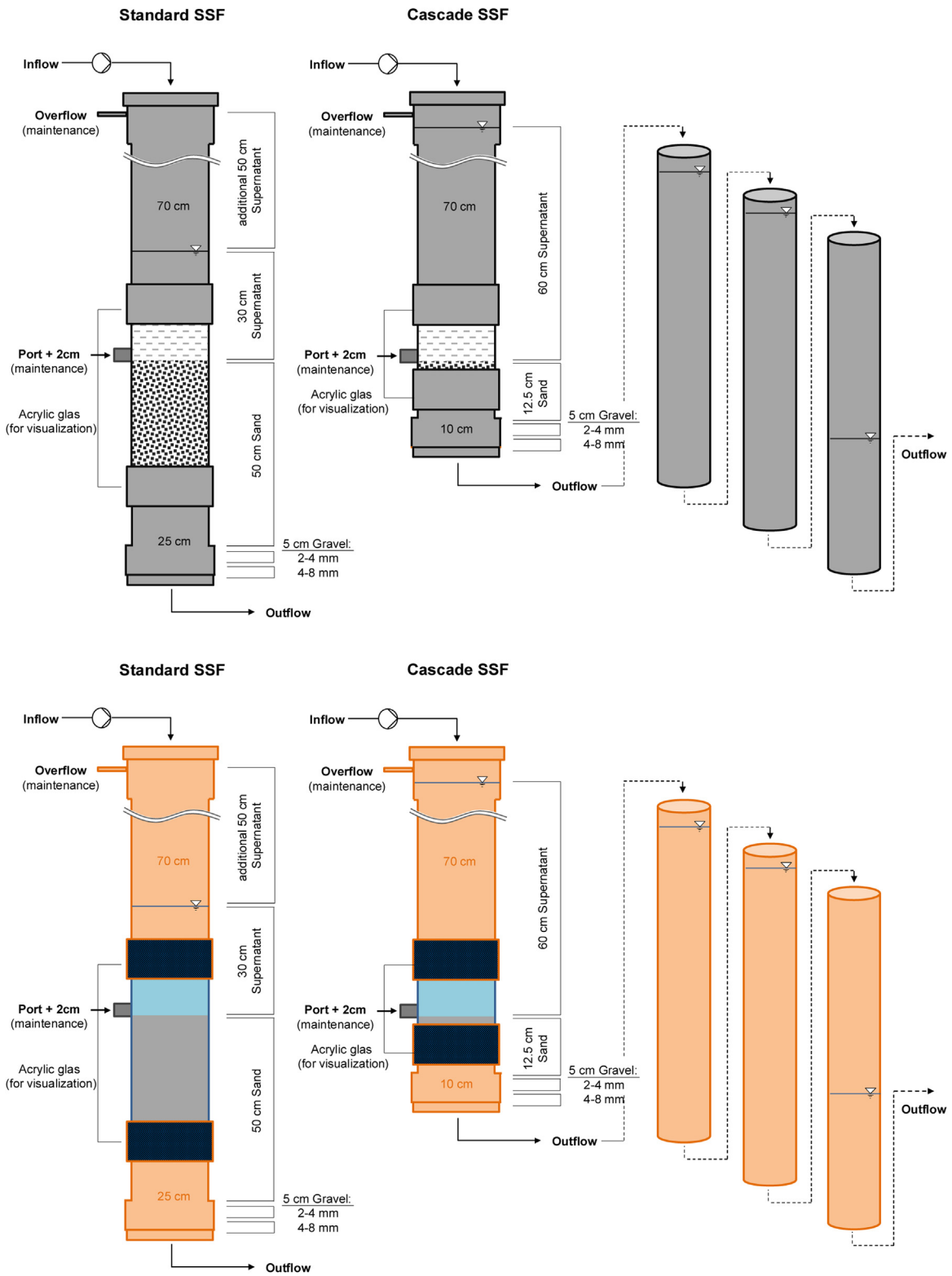

Fig. 1. Design and operation scheme of the standard SSF (***N***) and the cascade SSF (***C***).

tration); and entero-/adenoviruses (represented by somatic and F-specific RNA coliphages, as indicators for virus inactivation and removal; especially the latter are similar in size, shape and composition to many human enteric viruses) (Payment et al., 2003; Yates, 2007; Bauer et al., 2011).

2. Materials and methods

2.1. Secondary effluent: HSSF CW and WWTP Langenreichenbach

An HSSF CW with a bed depth of 50 cm was operated at the Langenreichenbach Ecotechnology Research Facility, 50 km northeast of Leipzig, Germany (51° 29′ 00″ N and 12° 54′ 00″ E). It was fed

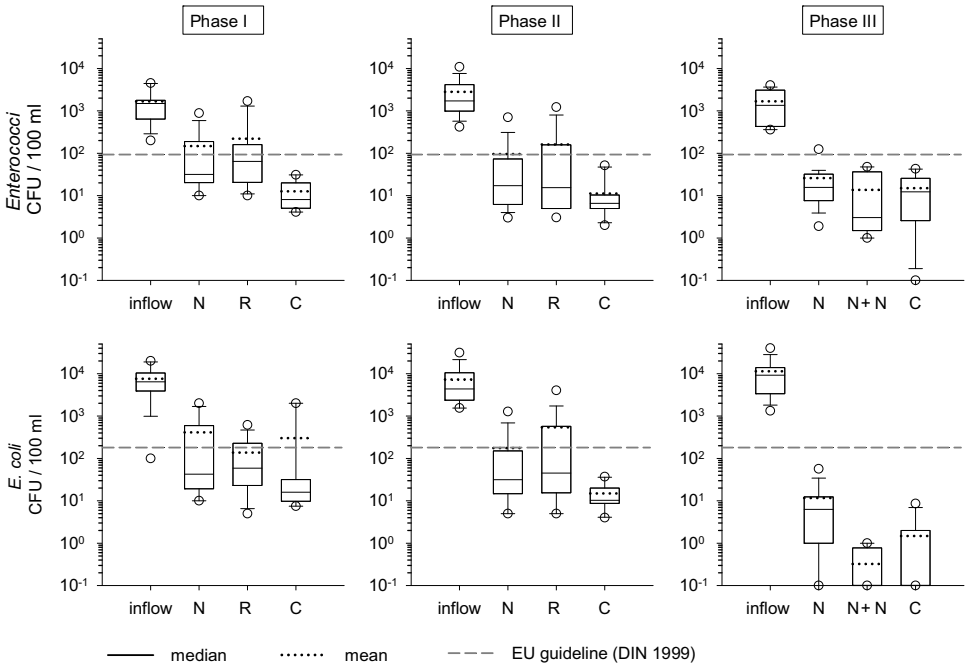

Fig. 2. SSF inflow and outflow concentrations of *Enterococci* and *E. coli* during the three experimental phases.For both parameters the time series (Phase I, II, III) showed no significant slope in a relevant order of magnitude, allowing for box plot presentations. Box plots settings: boxes comprise data within the first and third quartile with whiskers at 95th and 5th percentile; outliers are represented by open dots. For the graphical presentation in log 10 units, zero values were set to 0.1. *N*: standard SSF, *R*: recirculating SSF, *C*: rotating cascade, *N + N*: static cascade.

with domestic wastewater from a rural area (nominal HLR 36 l m^{-2} d^{-1}) pre-treated in a sedimentation tank. The feed was branched off from the inlet pipe of the local wastewater treatment plant (WWTP, 16,000 population equivalents). CW influent characteristics, set-up, operation and *E. coli* removal efficiency are described elsewhere (Headley et al., 2013; Nivala et al., 2013). HSSF CW effluent quality with respect to HPC, enterococci, *C. perfringens* spores and somatic coliphages was determined in order to fully characterize the typical influent for tertiary SSFs (Table 1). However, due to its lower variability in the effluent quality and year-round availability of large water volumes, secondary clarifier effluent (SCE) from the WWTP was used as SSF feed for the current investigation. While pathogen loads were generally lower in the SCE than in HSSF CW effluent (Table 1), they were in the same range as expected for efficiently operating secondary subsurface flow CWs. The concentrations of other wastewater constituents were below the thresholds of the German wastewater regulation for WWTP effluents (Table S1).

2.2. Pilot-scale SSFs

The experimental SSF plant was implemented on-site at the Langenreichenbach WWTP in spring 2013 and was operated in the dark in an isolated container. In winter, temperatures in the container were held above 5 °C by an electric fan heater. SCE from the WWTP was pumped to the container through an isolated pipe of approximately 25 m length, equipped with heating cables to prevent freezing (15 W/m, A. Rak Wärmetechnik, Germany). SSF feed was dosed from a permanently flushed tank (2.5 l min^{-1}) inside the container.

Three promising optimization approaches were tested against the standard SSF (***N***) for enhanced pathogen removal (Fig. 1): (i) an SSF with direct recirculation of 33% of the inflow water load (***R***); (ii) a cascade of two standard SSFs (***N + N***); and (iii) a rotating cascade comprising four shorter SSFs in series (***C***).

The SSFs were constructed using PVC pipes with an inner diameter of 19 cm. ***N***, ***R*** and ***N + N*** SSFs (two replicates each) contained a sand bed of 50 cm depth. The four ***C*** filters of the rotating cascade contained a sand bed of 12.5 cm each, resulting in the same total bed depth as for one long SSF (Fig. 1) but comprising four separate *Schmutzdecke* layers. The employed quartz sand was mixed using commercially available grain size fractions (Busch Quarz, Germany) according to the material achieving the highest faecal indicator removal in the study of Langenbach et al. (2009): d_{10} = 0.21; d_{60} = 0.36; d_{100} = 0.63, U (d_{60}/d_{10}) = 1.7 and porosity P = 0.38. All filter beds were supported by 5 cm of fine gravel (ø = 2–4 mm, P = 0.4) on top of 5 cm of coarse gravel (ø = 4–8 mm, P = 0.39). The filters were continuously fed with SCE using peristaltic pumps at hydraulic loading rates of 10 cm h^{-1} (Phase I) and 5–6.7 cm h^{-1} (Phase II and III). The permanent supernatant level of 30 cm was controlled by outflow weirs; the water was allowed to rise to a level of 70 cm before maintenance was performed (wet harrowing, approx. every 4–6 weeks). Ports were installed directly above the filter surface to allow removal of excess *Schmutzdecke* suspension.

For the filter cascade ***N + N***, effluent of filter 1 was fed to filter 2 by pumping, while for the rotating cascade ***C***, the 4 filters were installed with a height difference of 20 cm between each two consecutive SSFs to enable wastewater flow. With the given height difference, a permanent supernatant level of approx. 60 cm led to overflow into the next filter; only the at present last ***C*** filter was operated with a supernatant level of 30 cm. Periodic rotation of the wastewater recipient order was carried out using a pulley system to adjust the height of the filters.

2.3. Experiments and sampling

Operation of the experimental SSFs started in May 2013. The following experimental phases were investigated:

Phase 1 (July–November 2013): Operation of ***N***, ***R*** and ***C***; HLR: 10 cm h^{-1}; recirculation rate in ***R***: 33%; rotation cycle in ***C***: about every 3 weeks; 7–12 sampling events for all pathogen indicators.

Phase 2 (December 2013–May 2014): Operation of ***N***, ***R*** and ***C***; HLR: ***N*** and ***C*** 5 cm h^{-1}, ***R*** 6.7 cm h^{-1}; recirculation rate in ***R***: 33%;

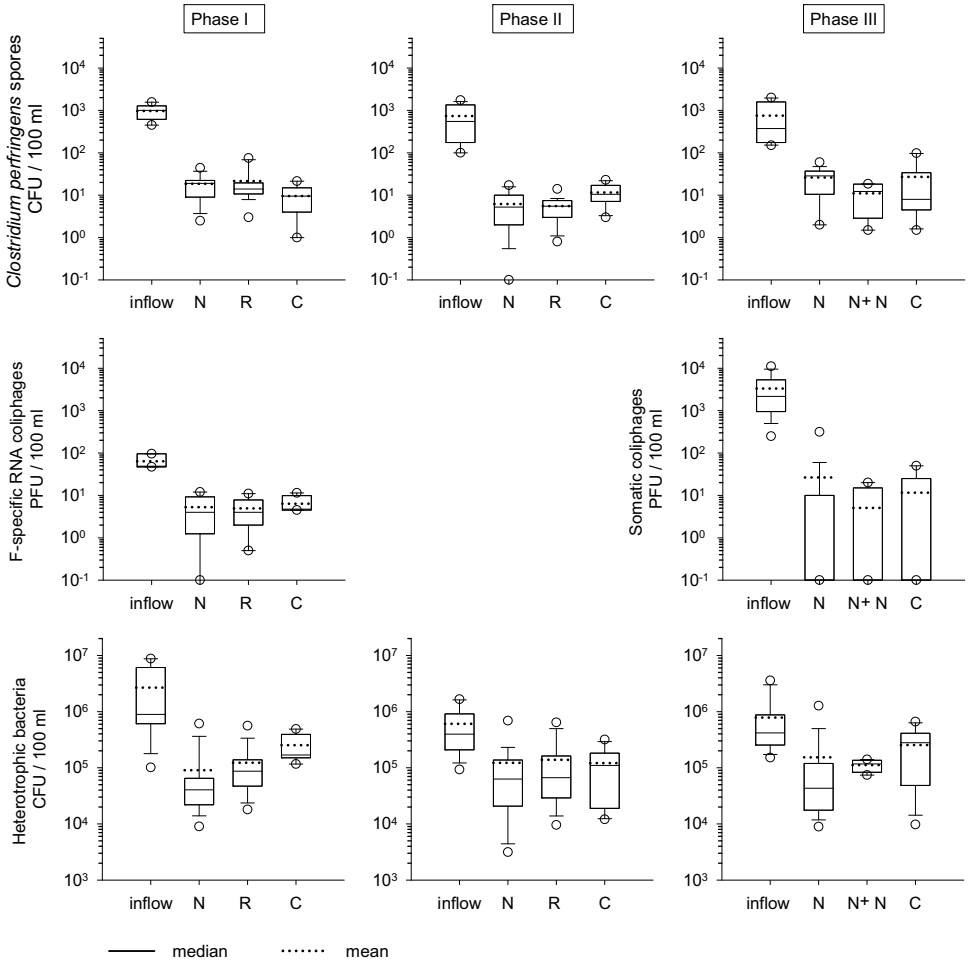

Fig. 3. SSF inflow and outflow concentrations of *C.perfringens* spores, heterotrophic bacteria and coliphages during the three experimental phases.For all parameters the time series (Phase I, II, III) showed no significant slope in a relevant order of magnitude, allowing for box plot presentations. Box plots settings: boxes comprise data within the first and third quartile with whiskers at 95th and 5th percentile; outliers are represented by open dots. For the graphical presentation in log 10 units, zero values were set to 0.1. *N*: standard SSF, *R*: recirculating SSF, *C*: rotating cascade, *N+N*: static cascade.

rotation cycle in ***C***: about every 3 weeks; 10–16 sampling events for all pathogen indicators.

Phase 3 (July–November 2014): Operation of ***N***, ***N+N*** and ***C***; HLR: 5 cm h^{-1}; rotation cycle in ***C***: every week; 10–15 sampling events for all pathogen indicators except F-specific RNA coliphages (3–5 sampling events), 2 sampling events for ***N+N***.

The HSSF CW was sampled from June to November 2014. For SSF und HSSF CW sampling, 500–1000 ml and 250–500 ml of water were taken from the inflow and outflow of the investigated systems depending on the necessary dilutions.

2.4. Methods for the determination of pathogen indicators

A suite of routine monitoring parameters to evaluate pathogen removal in wastewater treatment systems was established. The chosen indicators represent all important groups of microscopic pathogens present in wastewater:

Heterotrophic Plate Counts as an indicator for general bacterial load, according to Method 9215C (APHA/AWWA/WEF, 2012): Spread Plate method using Reasoner's 2A (R2A) agar, sample volume 0.1 ml, incubation for 48 h at 35 ± 0.5 °C;

E. coli representing coliform bacteria, according to method 9223 B (APHA/AWWA/WEF, 2012): Defined Substrate Technology using Colilert with QuantiTray/QuantiTray2000 (IDEXX Laboratories, USA), Most Probable Number (MPN) multi-well procedure, sample volume 100 ml, incubation for 24 h at 35 ± 0.5 °C;

Enterococci representing more resistant faecal bacteria, according to method 9230 D (APHA/AWWA/WEF, 2012): Defined Substrate Technology using Enterolert-E with QuantiTray/QuantiTray2000 (IDEXX Laboratories, USA), MPN multi-well procedure, sample volume 100 ml, incubation for 24 h at 41 ± 0.5 °C;

Presumptive *C. perfringens* spores [i.e. spores of anaerobic *Clostridia* growing on selective Sulphite Polymyxin Sulphadiazine (SPS) agar] as proxy for (oo)cysts of protozoan parasites (e.g. *Giardia* and *Cryptosporidium* ssp.), according to BS EN ISO 7937 (BSI, 2004), Bonadonna et al. (2002), DIN EN 26 461/ISO 6461-2 (DIN, 1993), and Tandoi and Levantesi (personal communication, 2013): Pour tube (sample volume 2–25 ml) and membrane filter (sterile 0.45 μm cellulose nitrate/mixed cellulose ester filters, ø = 47 mm, sample volume 100 ml) methods using SPS agar, anaerobic incubation (AnaeroGen system, Oxoid) for 20 ± 4 h at 36 ± 1 °C;

F-specific RNA coliphages as proxy for human entero- and adenoviruses, according to methods 9224C and F (APHA/AWWA/WEF, 2012): Membrane filter method (sterile 0.45 μm mixed cellulose ester filters, ø = 47 mm, sample volume 100–250 ml) using *E. coli* Famp (ATCC700891) and reference phage MS2 (ATCC15597-B1), trypton agar, incubation overnight at 36.5 ± 2 °C;

Somatic coliphages as a proxy for human entero- and adenoviruses according to method ISO 10705-2 (ISO, 2000): Double-layer agar method using nalidixic acid resistant strain *E. coli* WG5

Table 2
Kruskal-Wallis one-way analysis of variance on ranks ($P \leq 0.001$) with post-hoc pairwise multiple comparisons (Dunn's Method; $P \leq 0.05$) of all SSF configurations was performed in SigmaPlot (version 13.0).The table shows only significant comparisons. Filter configuration **X** results in significantly higher removal than filter configuration **Y** for the given pathogen parameter; only significant results are listed. The following abbreviations are used: HLR = hydraulic loading rate; ***N*** = standard treatment; ***N-N*** = static cascade; ***R*** = recirculating SSF; ***C*** = rotating cascade; Recirculation rate = RR; Rotation cycle = RC; *Cps* = *C. perfringens* spores.

					Y				
	SSF				N			R	
		Phase			1	2	3	1	2
			HLR [cm h^{-1}]		10	5	5	10	6.6
				specification	–	mth 1–6	mth 7–11	RR: 33%	RR: 33%
X	N	I	10	–		–	–	–	–
		II	5	mth 1–6	*Cps*		*Cps*	*Cps*	–
		III	5	mth 7–11	*E. coli*	*E. coli*		*E. coli*	*E. coli*
	N + N	III	5	–	*E. coli*	*E. coli*	–	*E. coli*	*E. coli*
	R	I	10	RR: 33%	–	–	–		–
		II	6.6	RR: 33%	*Cps*	–	*Cps*	*Cps*	
	C	I	10	RC: triweekly	–	–	–	–	–
		II	5	RC: triweekly	–	–	–	–	–
		III	5	RC: weekly	*E. coli*	*E. coli*	–	*E. coli*	–

(ATCC 700078) and reference phage φX174 (ATCC 13706-B1), Modified Scholtens'Agar, incubation for 18 ± 2 h at 36 ± 2 °C.

2.5. Methods for the determination of physico-chemical parameters

In order to characterize the SSF influent (SCE), a suite of physico-chemical parameters was measured on a routine basis (Supporting Information, Table S1): Dissolved Organic Carbon (DOC) and Total Nitrogen (TN) on a Multi N/C® 2100 TOC/TN_b Analyzer (Analytik Jena, Germany) after filtration through a 0.45 μm syringe filter; NH_4^+, NO_2^- and NO_3^- using Merck photometric tests (1.00683.0001, 1.14776.0001, and 1.09713.0001 respectively) and a Merck Spectroquant Nova60 photometer; and pH using a Sentix 41 pH electrode (WTW, Germany) and a pH526 pH meter (WTW, Germany).

3. Results and discussion

Figs. 2 and 3 summarize the overall removal of the pathogen indicators after passage of the four differently designed SSFs during the three operational phases. All SSF configurations led to a considerable removal of the monitored indicator numbers.

When assessing compliance of treated wastewater with irrigation water standards, the two main bacterial indicator parameters *E. coli* and enterococci are of crucial importance. If no safe compliance can be achieved with a given treatment system, its application – at least in Europe – is impracticable, regardless of the removal performance reached for other parameters. For the additional water quality parameters *C. perfringens* spores, F-specific RNA and somatic coliphages, and heterotrophic bacteria, no respective target values are given in the established irrigation water guidelines. Nevertheless, the removal of additional indicators for protozoan (oo)cysts and human enteroviruses in the tested SSF configurations was investigated because both pathogen groups can lead to serious illness when present on crops for (raw) human consumption. The behaviour of aerobic heterotrophs in the tested systems was determined in order to estimate the removal of total bacterial load.

3.1. Escherichia coli *and enterococci*

E. coli and enterococci are used as faecal indicator bacteria to assess microbiological impairment of water throughout the world (Wang et al., 2013). These microorganisms are generally associated with faeces from humans and other warm-blooded animals, while *Enterococcus faecium* and *Enterococcus faecalis* are more likely human-specific (Boehm and Sassoubre, 2014). *E. coli* is widely used as a treatment performance indicator, even though it is more sensitive to disinfection than many pathogens. Enterococci are recommendable additional performance indicators for removal of enteric (bacterial) pathogens due to their higher resistance to stress and drying, as well as generally longer survival times (Payment et al., 2003; Yates, 2007). Their occurrence in water may also signal the presence of enteric pathogens such as hepatitis A virus (Simpson et al., 2002) and *Salmonella* (Ashbolt et al., 2001; Boehm and Sassoubre, 2014). However, according to Field and Samadpour (2007) the correlation of *E. coli* and enterococci detection in the environment with detection of many pathogens (*Salmonella spp*., *Campylobacter* spp., *Cryptosporidium* and *Giardia* spp., human enteroviruses including adenoviruses and coliphages) is questionable. Removal of *E. coli* and enterococci occurs mainly via biological processes such as predation and infection by lytic bacteriophages. Other mechanisms include environmental stresses such as light and dark inactivation (Haig et al., 2015; Boehm and Sassoubre, 2014). Individuals of both genera can enter a viable but non-culturable (VBNC) state, and they have been reported to grow under certain circumstances outside their typical hosts, e.g. in sands, soils and sediments (Field and Samadpour, 2007; Boehm and Sassoubre, 2014).

For *E. coli* and enterococci, the obtained mean reduction in the SSF units was in the range of 1.1–4.7 and 0.9–2.4 log units, respectively. However, only the rotating cascade ***C*** (Phases II and III) and the static cascade ***N + N*** (Phase III) complied with European guideline values for both *E. coli* (200 CFU 100 ml^{-1}) and enterococci (100 CFU 100 ml^{-1}), achieving log removals of 2.7, 3.9 and 4.7 for *E. coli* and 2.4, 2.1 and 2.1 for enterococci, respectively. Overall, observed *E. coli* and enterococci removal in this study was within the range reported previously for standard SSF (1.9–4.1 and 0.7–3.7 log units for *E. coli* and enterococci, respectively; Mälzer, 2005; Langenbach et al., 2009; Bauer et al., 2011; Pfannes et al., 2015). Considerably higher removal efficiencies for *E. coli* compared to enterococci, especially for ***C*** and ***N + N*** configurations in Phases II and III, may reflect the higher resistance of enterococci against stress (Payment et al., 2003).

Based on the present outflow load data, ***C*** is the most reliable SSF configuration (Fig. 2; narrow widths of the data boxes representing ***C***) even at the beginning of operation (Phase I). The similarity in performance of the rotating cascade ***C*** and the static cascade

N+N is supported by the statistical analysis (see Table 2): in most cases, outflow values are significantly lower for ***C*** with weekly rotation (Phase III) and for ***N+N*** than for the conventional filter ***N*** and the recirculating filter ***R***. Even though for the configuration ***N+N*** only a few data points exist, the simple cascade of two standard SSFs appears as a feasible treatment option that is less costly and time-consuming in terms of maintenance compared to the rotating cascade ***C***.

In contrast, the standard filter ***N*** showed improved removal efficiency only after a longer operation period (Phase III compared to Phase II) (see Fig. 2). For enterococci, the data box became narrower and the mean value met the limit of 100 CFU 100 ml^{-1} in Phase III. For *E. coli* the same trend was observed, and removal in ***N*** in Phase III was significantly higher than removal in ***N*** and ***R*** in Phases I and II (see Table 2). Even longer operation periods may further strengthen this trend for enterococci, resulting in 100% compliance with the limit. The fact that removal in ***N*** improved over time and was no longer significantly different from removal in ***C*** in Phase III suggests that four *Schmutzdecke* layers may not necessarily outperform a single *Schmutzdecke* after long-term build-up and equilibration under static operation conditions. It remains to be investigated whether significant advantages of ***C*** over ***N*** continue to exist after extended operation periods. The observed increased removal efficiency for ***N*** filters at longer operation times is a strong indicator for active biological mechanisms as the main removal processes for *E. coli* and enterococci, given that the microbial community in the dirt layer had more time to develop. This corresponds with the results of Haig et al. (2015) who investigated top-down trophic interactions, identifying protozoan grazing responsible for the majority of the *E. coli* removal in SSF, and Pfannes et al. (2015) who also highlighted the contribution of biological factors to *E. coli* removal in the *Schmutzdecke*.

3.2. Clostridium perfringens *spores*

C. perfringens spores ($ø \leq 1$ μm, Novak et al., 2003) are dormant bacterial cells highly resistant to heat, drying and chemical disinfectants and used as proxy for protozoan (oo)cysts ($ø \approx 5$ μm, Schijven et al., 2003). Compared to (oo)cysts, *C. perfringens* spores are retained less by physical straining but are more efficiently adsorbed, and are more resistant to predation and inactivation. Thus, they represent a conservative surrogate for (oo)cysts (Schijven et al., 2003; Hijnen et al., 2004, 2007), even though spore and (oo)cyst removal are not always clearly correlated in SSF (Heller et al., 2007). Given that most CW systems show only moderate removal of *C. perfringens* spores and protozoan (oo)cysts (≤2 log units; Redder et al., 2010; Abreu-Acosta and Vera, 2011; Morató et al., 2014), post-treatment e.g. in SSFs is required in order to provide safe irrigation water.

The mean concentration of *C. perfringens* spores in the SSF inflow was 7.4–9.7 × 10^2 CFU 100 ml^{-1}. The investigated SSFs achieved a mean removal of 1.4–2.1 log units (median 1.1–2) with no obvious impact of the type of filter configuration (Fig. 3). The removal of *C. perfringens* spores in continuous SSF for wastewater disinfection has not previously been reported in the literature to the best of our knowledge. In intermittently loaded recirculating SSF with recirculation tanks, spore removal was 1.2–2.3 log units (Gold et al., 1992). Further findings refer to drinking water treatment with low and variable inflow loads, and may not be fully comparable to our study. A spiking experiment with *C. perfringens* spores in full-scale drinking water filters showed a reduction of 2–3 log units, while a removal rate of 3.9 log units was achieved in continuously operated systems (Hijnen et al., 2004, 2007). Generally, bacterial spores may survive in the environment for many years. In SSF, *C. perfringens* spores are mainly removed by physical straining and adsorption (Schijven et al., 2003; Hijnen et al., 2004). Predation by zooplankton was shown for *Cryptsporidium* oocysts, but is probably less relevant for *Clostridia* spores (Hijnen et al., 2007). Davies et al. (1995) found that *C. perfringens* spores were unaffected by predators in marine and freshwater sediments, and that no considerable inactivation occurred over 50 days. Inactivation rates of spores were estimated as low as 0.003–0.005 log d^{-1} in SSF and river water (Medema et al., 1997; Hijnen et al., 2007). Environmental inactivation of *Cryptosporidium* oocysts is considerably higher with 0.015–0.022 and 0.01 log d^{-1} in soil and surface water, respectively (Davies et al., 2005; King et al., 2005).

Considering straining and adsorption as main removal mechanisms, higher elimination would be expected for ***R*** and ***N+N*** filters than for ***N*** and ***C*** filters due to a longer filter distance and therefore higher capacity (***N+N***) or more opportunity (***R***) for retention. Statistical analysis indicates that at a lower HLR, ***R*** filters may indeed show an advantage over standard ***N*** filters (Table 2). ***R*** filters with HLR = 5 cm h^{-1} performed significantly better than ***R*** filters with HLR = 10, and better than ***N*** filters with HLR = 5 (Phase III) and 10. Moreover, statistics showed that at low HLR, ***N*** performed better in Phase II than in Phase III. This may be due to a reduction of available adsorption sites over time and insufficient inactivation of adsorbed spores, thus suggesting sorption as a dominant removal process rather than predation. The fact that no improvement of removal was observed in the ***C*** filters substantiates this interpretation. A dominant contribution of predation would have led to higher removal in ***C*** due to several *Schmutzdecke* layers. Schijven et al. (2003) reported that adsorption of spores was mainly reversible, eventually leading to complete breakthrough if no inactivation occurs. This could not be conclusively verified by our results; either the adsorption capacity had not yet been reached after 1½ years of operation, or at least some predation/inactivation of adsorbed spores occurred. The variability of datasets was lower for *C. perfringens* compared to *E. coli* and enterococci data, thus filter performance was more predictable (Figs. 2 and 3). The static filter cascade ***N+N*** did not show the expected improvement of removal, but the data set is relatively small and the approach needs to be further investigated (Fig. 3).

3.3. Coliphages

F-specific RNA coliphages, infecting bacterial cells through F-pili, were suggested to represent human enteroviruses especially well (Payment et al., 2003; Nieminski et al., 2000; Grabow 2004; Bauer et al., 2011) and are thus a suitable conservative surrogate for the latter in the testing of treatment systems (Schijven et al., 2003). Their removal was studied in ***N***, ***R*** and ***C*** filters at HLR = 10 cm h^{-1}. Observed removal rates were very similar for all investigated systems with a mean and median log removal of 1.0–1.1 at mean inflow concentrations of 64 Plaque Forming Units (PFU) 100 ml^{-1}. For temperatures above 8 °C, removal rates for this type of phage reported in the literature tend to be higher, and range from 1.4–3.5 for MS2 bacteriophages (Poynter and Slade, 1977; Hijnen et al., 2004; Anderson et al., 2009; Schijven et al., 2013; Young-Rojanschi and Madramootoo, 2014). The consistently lower log removal observed in our experiments could be due to the lower temperatures in part of Phase I (autumn/winter 2013), which have been shown elsewhere to decrease MS2 removal (Anderson et al., 2009; Schijven et al., 2013), or to the lower inflow load compared to other studies.

Somatic coliphages are a more diverse group of *E. coli* bacteriophages infecting their hosts via the cell wall. They have been shown to be a suitable surrogate for virus removal (Nieminski et al., 2000). The removal of somatic coliphages in ***N***, ***N+N*** and ***C*** filter configurations was investigated at a HLR of 5 cm h^{-1}. At a mean inflow load of 3.5 × 10^3 PFU 100 ml^{-1}, the pilot systems achieved mean removal of 2.4–2.8 log units. Bauer et al. (2011) have reported similar removal efficiencies for standard SSF of 2.3–3.2 log units over a

wide range of HLRs, but with a longer filter passage of 0.9 m. Higher removal of somatic compared to F-specific RNA coliphages can be explained not only by the higher resistance of the F-specific RNA coliphages against inactivation during treatment (Payment et al., 2003), but also by the lower HLR (Anderson et al., 2009) and a potentially increased performance after long operation time.

In the literature, different mechanisms and sand filter zones important for removal of viruses have been reported. In a study by Schijven et al. (2004), adsorption was the most significant factor in elimination of the bacteriophage MS2. Elliot et al. (2011) found bacteriophage reduction in SSF also due to microbial activity, probably via excretion of proteolytic enzymes and grazing by protozoa (e.g. Pinheiro et al., 2007). Hijnen et al. (2004) showed that the *Schmutzdecke* did not influence bacteriophage removal considerably, attributing this finding to the low impact of physical straining and biological inactivation mechanisms. In contrast, Dizer et al. (2004) reported a much better retention of an F-specific coliphage in an SSF with *Schmutzdecke* compared to one without. Our results showed that no somatic and F-specific RNA coliphage removal optimization was achieved by the ***C*** filter configuration. Thus, a positive effect of several active *Schmutzdecke* layers and therefore enhanced virus removal through microbial activity could not be verified. An increased removal in ***R*** due to more opportunity for adsorption and inactivation could also not be corroborated by our results. The slightly elevated somatic coliphage removal efficiency of the ***N+N*** configuration may reflect its doubled sand bed length and therefore adsorption capacity; however, these results can only be regarded as preliminary due to the small number of samples from ***N+N***. Overall, the tested SSFs showed excellent removal of somatic coliphages. With outflow values often around the detection limit of 10 PFU 100 ml^{-1} and constrained by the sample volume that could be taken, it may be difficult to detect differences between filter types even if there were any. In accordance with this, statistical differences between filter configurations could be detected neither for F-specific nor for somatic coliphages.

3.4. Heterotrophic Plate Counts

HPC can be used as a parameter for general bacterial load of waters. While of no demonstrated sanitary significance, HPC may be useful to assess the efficiency of water treatment and monitor the general status of the treatment system. Strong changes in counts can indicate negative developments such as microbial growth (Payment et al., 2003). With mean inflow loads of 6.1×10^5–2.7×10^6 CFU 100 ml^{-1}, the tested filter configurations achieved mean removal of 0.5–1.5 log (medians 0.2–1.3 log). Removal was highest in all filter configurations in Phase I with the higher HLR. Lower removal of heterotrophic bacteria as compared to *E. coli* has been observed before (e.g. Ahammed and Chaudhuri, 1996) and is apparently due to microbe-specific elimination mechanisms in SSF (Pfannes et al., 2015). The ***C*** system tended to remove less HPC than the other configurations in Phases I and III, while in Phase II removal was similar for all tested filter types. The ***C*** filters contain higher water volumes above the *Schmutzdecke*. The associated longer retention time may allow a fraction of the heterotrophic bacteria to utilize residual metabolic energy sources and thus experience lower decay or even growth.

4. Conclusions

All investigated filter configurations achieved substantial mean removal for *E. coli* (≤4.7 log), enterococci (≤2.4 log), *C. perfringens* spores (≤2.1 log), coliphages (≤2.8 log) and HPC (≤1.5 log) with the tested operational conditions.

Only rotating (***C***) and static (***N+N***) cascade systems concurrently complied with European irrigation water standards for both *E. coli* and enterococci at HLR = 5 cm h^{-1}. The best performance was reached by the cascade with weekly rotation, but preliminary results indicated that the static cascade may be a promising, technically simpler alternative.

During an operation period of up to 1 ½ years, the standard filter (***N***) showed considerable improvement of removal efficiency for *E. coli* and enterococci. Hence, it needs to be clarified whether advantages of cascade over standard systems persist after years of operation.

A good *C. perfringens* spore removal performance was observed for all investigated SSF systems, indicating presumably even better protozoan (oo)cyst removal; recirculating filters (***R***) showed significant advantage over standard filters (***N***) at low HLR. The dominant role of sorption and negligible contribution of active biological mechanisms for spore removal was indicated by: (i) decreased efficiency in ***N*** and ***C*** filters at long operation times, and (ii) no impact of several *Schmutzdecke* layers on removal performance. Thus, long-term removal performance of SSF for spores and (oo)cysts remains questionable.

Somatic coliphages were reduced to concentrations close to the detection limit in ***N***, ***N+N*** and ***C***, and therefore potential differences between filters could not be determined. F-specific RNA coliphage removal was ~1.1 log for ***N***, ***R*** and ***C*** filters and thus lower than reported in the literature.

Mean removal efficiency of HPC in the investigated filters was low (0. 5–1.5 log) compared to other studies.

As compliance with irrigation water standards is mandatory for wastewater reuse, the cascade systems ***C*** and ***N+N*** are the most promising options for potential users. From a practical perspective static cascade systems are easier to implement and maintain.

Acknowledgements

The present study was funded by the European Commission (Grant Agreement FP7-KBBE-2012-6-311933) in the framework of the project “Integrating bio-treated wastewater reuse and valorization with enhanced water use efficiency to support the Green Economy in EU and India (Water4Crops)”, and was co-financed by the Helmholtz Center for Environmental Research UFZ. We thank Jaime Nivala for granting us access to the Ecotechnology Research Facility in Langenreichenbach and Katy Bernhard for taking constructed wetland samples. Astrid Birnbaum, Kerstin Ethner, Ines Mäusezahl, Franziska Reincke, Nancy Rümmelein, Desiree Schmidt, Anja Taubert and Uwe Kappelmeyer are acknowledged for their valuable support in the field and laboratory.

Our heartfelt gratitude goes to Peter Kuschk (†2014) for his assistance during conception and implementation of the SSF pilot system, and for his professional and personal advice and practical support over many years. We miss him deeply.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ecoleng.2016.06.068.

References

ANZECC, 2000. Australian and New Zealand Guidelines for Fresh and Marine Water Quality, 3 Primary Industries. Australian and New Zealand Environment and Conservation Council and the Agriculture and Resource Management Council of Australia and New Zealand, Canberra, Australia.

APHA/AWWA/WEF, 2012. In: Rice, E.W., Baird, R.B., Eaton, A.D., Clesceri, L.S. (Eds.), Standard Methods for the Examination of Water and Wastewater. , 22nd

edition. American Public Health Association, American Water Works Association, Water Environment Federation.
Abou-Elela, S.I., Golinelli, G., El-Tabl, A.S., Hellal, M.S., 2014. Treatment of municipal wastewater using horizontal flow constructed wetlands in Egypt. Water Sci. Technol. 69, 38–47.
Abreu-Acosta, N., Vera, L., 2011. Occurrence and removal of parasites enteric bacteria and faecal contamination indicators in wastewater natural reclamation systems in Tenerife-Canary Islands, Spain. Ecol. Eng. 37, 496–503.
Adin, A., 2003. Slow granular filtration for water reuse. Water Sci. Technol.: Water Supply 3, 123–130.
Ahammed, M.M., Chaudhuri, M., 1996. Sand-based filtration/adsorption media. AQUA 45, 67–71.
Anderson, W.B., DeLoyde, J.L., Van Dyke, M.I., Huck, P.M., 2009. Influence of design and operating conditions on the removal of MS2 bacteriophage by pilot-scale multistage slow sand filtration. J. Water Supply Res. Technol. 58, 450–462.
Ashbolt, N.J., Grabow, W.O.K., Snozzi, M., 2001. Indicators of microbial water quality. In: Fewtrell, L., Bartram, J. (Eds.), Water Quality: Guidelines, Standards and Health. World Health Organization (WHO), IWA Publishing, London, UK.
BSI, 2014. BS EN ISO 7937: Microbiology of Food and Animal Feeding Stuffs Horizontal Method for the Enumeration of *Clostridium perfringens* – Colony-count Technique. The British Standards Institution, London, UK.
Bauer, R., Dizer, H., Graeber, I., Rosenwinkel, K.-H., López-Pila, J.M., 2011. Removal of bacterial faecal indicators, coliphages and enteric adenoviruses from waters with high faecal pollution by slow sand filtration. Water Res. 45, 439–452.
Boehm, A.B., Sassoubre, L.M., 2014. Enterococci as indicators of environmental fecal contamination. In: Gilmore, M.S., Clewell, D.B., Ike, Y., et, al. (Eds.), Enterococci: From Commensals to Leading Causes of Drug Resistant Infection. Massachusetts Eye and Ear Infirmary Boston http://www.ncbi.nlm.nih.gov/books/NBK190421/.
Bonadonna, L., Briancesco, R., D'Angelo, A.M., Marini, R., 2002. Rapporti ISTISAN 02/8 *Clostridium Perfringens* as an Environmental Pollution Indicator and Its Hygienic Role (in Italian). Istituto Superiore di Sanità, Rome, Italy.
Christou, A., Maratheftis, G., Eliadou, E., Michael, C., Hapeshi, E., Fatta-Kassinos, D., 2014. Impact assessment of the reuse of two discrete treated wastewaters for the irrigation of tomato crop on the soil geochemical properties, fruit safety and crop productivity. Agric. Ecosyst. Environ. 192, 105–114.
DIN, 1993. DIN EN 26 461/ISO 6461-2: Water Quality; Detection and Enumeration of the Spores of Sulfite-reducing Anaerobes (clostridia), Part 2: Method by Membrane Filtration (in German). Deutsches Institut für Normung e.V., Beuth Verlag Berlin, Germany.
DIN, 1999. DIN 19650: Irrigation – Hygienic Concerns of Irrigation Water (in German). Deutsches Institut für Normung e.V., Beuth Verlag Berlin, Germany.
Davies, C.M., Long, J.A.H., Donald, M., Ashbolt, N.J., 1995. Survival of fecal microorganisms in marine and freshwater sediments. Appl. Environ. Microbiol. 61, 1888–1896.
Davies, C.M., Altavilla, N., Krogh, M., Ferguson, C.M., Deere, D.A., Ashbolt, N.J., 2005. Environmental inactivation of *Cryptosporidium* oocysts in catchment soils. J. Appl. Microbiol. 98, 308–317.
Decamp, O., Warren, A., 2000. Investigation of *Escherichia coli* removal in various designs of subsurface flow wetlands used for wastewater treatment. Ecol. Eng. 14, 293–299.
Dizer, H., Grützmacher, G., Bartel, H., Wiese, H.B., Szewzyk, R., López-Pila, J.M., 2004. Contribution of the colmation layer to the elimination of coliphages by slow sand filtration. Water Sci. Technol. 50, 211–214.
Ellis, K.V., 1987. Slow sand filtration as a technique for the tertiary treatment of municipal sewages. Water Res. 21, 403–410.
Farooq, S., Alyousef, A.K., 1993. Slow sand filtration of secondary effluent. J. Environ. Eng.-ASCE 119, 615–630.
Field, K.G., Samadpour, M., 2007. Fecal source tracking, the indicator paradigm, and managing water quality. Water Res. 41, 3517–3538.
García, J.A., Paredes, D., Cubillos, J.A., 2013. Effect of plants and the combination of wetland treatment type systems on pathogen removal in tropical climate conditions. Ecol. Eng. 58, 57–62.
Ghermandi, A., Bixio, D., Traverso, P., Cersosimo, I., Thoeye, C., 2007. The removal of pathogens in surface-flow constructed wetlands and its implications for water reuse. Water Sci. Technol. 56, 207–216.
Gold, A.J., Lamb, B.E., Loomis, G.W., Boyd, J.R., Cabelli, V.J., McKiel, C.G., 1992. Wastewater renovation in buried and recirculating sand filters. J. Environ. Qual. 21, 720–725.
Grabow, W.O.K., 2004. Bacteriophages: update on application as models for viruses in water. Water SA 27, 251–268.
Haig, S.-J., Schirmer, M., D'Amore, R., Gibbs, J., Davies, R.L., Collins, G., Quince, C., 2015. Stable-isotope probing and metagenomics reveal predation by protozoa drives *E. coli* removal in slow sand filters. ISME J. 9, 797–808.
Headley, T., Nivala, J., Kassa, K., Olsson, L., Wallace, S., Brix, H., van Afferden, M., Müller, R., 2013. *Escherichia coli* removal and internal dynamics in subsurface flow ecotechnologies: effects of design and plants. Ecol. Eng. 61B, 564–574.
Healy, M.G., Rodgers, M., Mulqueen, J., 2007. Performance of a stratified sand filter in removal of chemical oxygen demand, total suspended solids and ammonia nitrogen from high-strength wastewaters. J. Environ. Manage. 83, 409–415.
Heller, L., Cardoso Martins Vieira, M.B., Alves de Brito, L.L., Pedrosa Salvador, D., 2007. Association between the concentration of protozoa and surrogates in effluents of the slow sand filtration for water treatment. Braz. J. Microbiol. 38, 337–345.
Hijnen, W.A.M., Schijven, J.F., Bonné, P., Visser, A., Medema, G.J., 2004. Elimination of viruses, bacteria and protozoan oocysts by slow sand filtration. Water Sci. Technol. 50, 147–154.
Hijnen, W.A.M., Dullemont, Y.J., Schijven, J.F., Hanzens-Brouwer, A.J., Rosielle, M., Medema, G., 2007. Removal and fate of *Cryptosporidium parvum*, *Clostridium perfringens* and small-sized centric diatoms (*Stephanodiscus hantzschii*) in slow sand filters. Water Res. 41, 2151–2162.
ISO, 2000. ISO 10705-2(E) Water Quality – Detection and Enumeration of Bacteriophages – Part 2: Enumeration of Somatic Coliphages. International Organization for Standardization, Geneva, Switzerland.
Italian Decree, 2003. Regulating Technical Standards for Wastewater Reuse. Decreto Ministeriale Nr. 185, 12 June 2003. Il Ministro dell' Ambiente e dellatutela del territorio, I Ministri delle politiche agricole e forestali, delle attività produttive e della salute, Rome, Italy.
Kader Yettefti, I., Aboussabiq, F.E., Etahiri, S., Malamis, D., Assobhei, O., 2013. Slow sand filtration of effluent from an anaerobic denitrifying reactor for tertiary treatment: a comparable study, using three Moroccan sands. Carpath. J. Earth Environ. Sci. 8 (3), 207–218.
Kadewa, W.W., Le Corre, K., Pidou, M., Jeffrey, P.J., Jefferson, B., 2010. Comparison of grey water treatment performance by a cascading sand filter and a constructed wetland. Water Sci. Technol. 62, 1471–1478.
Keraita, B., Drechsel, P., Konradsen, F., Vreugdenhil, R.C., 2008. Potential of simple filters to improve microbial quality of irrigation water used in urban vegetable farming in Ghana. J. Environ. Sci. Health A 43, 749–755.
King, B.J., Keegan, A.R., Monis, P.T., Saint, C.P., 2005. Environmental temperature controls *Cryptosporidium* oocyst metabolic rate and associated retention of infectivity. Appl. Environ. Microbiol. 71, 3848–3857.
Langenbach, K., Kuschk, P., Horn, H., Kästner, M., 2009. Slow sand filtration of secondary clarifier effluent for wastewater reuse. Environ. Sci. Technol. 43, 5896–5901.
Langenbach, K., Kuschk, P., Horn, H., Kästner, M., 2010. Modeling of slow sand filtration for disinfection of secondary clarifier effluent. Water Res. 44, 159–166.
Logsdon, G.S., Kohne, R., Abel, S., LaBonde, S., 2002. Slow sand filtration for small water systems. J. Environ. Eng. Sci. 1, 339–348.
Mälzer, H.-J., 2005. R&D in the Field of Water Supply and Waste Water Treatment Under Regional Conditions Part I: Drinking Water, vol. 2: Recommendations. DVGW Technologiezentrum Wasser, Karlsruhe, Germany.
Masi, F., Conte, G., Lepri, L., Martellini, T., Del Bubba, M., 2004. Endocrine Disrupting Chemicals (EDCs) and pathogens removal in a hybrid CW system for a tourist facility wastewater treatment and reuse. Avignon (France) In: Proceedings of the 9th IWA International Conference on Wetland Systems for Water Pollution Control, Vol. 2, pp. 461–468.
Medema, G.J., Bahar, M., Schets, F.M., 1997. Survival of *Cryptosporidium parvum*, *Escherichia coli*, faecal enterococci and *Clostridium perfringens* in river water: influence of temperature and autochthonous microorganisms. Water Sci. Technol. 35, 249–252.
Miranda, N.D., Oliveira, E.L., Silva, G.H.R., 2014. Study of constructed wetlands effluent disinfected with ozone. Water Sci. Technol. 70, 108–113.
Morató, J., Codony, F., Sánchez, O., Pérez, L.M., García, J., Mas, J., 2014. Key design factors affecting microbial community composition and pathogenic organism removal in horizontal subsurface flow constructed wetlands. Sci. Total Environ. 481, 81–89.
Nieminski, E.C., Bellamy, W.D., Moss, L.R., 2000. Using surrogates to improve plant performance. J. AWWA 92, 67–78.
Nivala, J., Headley, T., Wallace, S., Bernhard, K., Brix, H., van Afferden, M., Müller, R.A., 2013. Comparative analysis of constructed wetlands The design and construction of the ecotechnology research facility in Langenreichenbach, Germany. Ecol. Eng. 61, 527–543.
Norton-Brandão, D., Scherrenberg, S.M., van Lier, J.B., 2013. Reclamation of used urban waters for irrigation purposes – a review of treatment technologies. J. Environ. Manage. 122, 85–98.
Novak, J.S., Juneja, V.K., McClane, B.A., 2003. An ultrastructural comparison of spores from various strains of *Clostridium perfringens* and correlations with heat resistance parameters. Int. J. Food Microbiol. 86, 239–247.
Payment, P., Waite, M., Dufour, A., 2003. Chapter 2?Introducing parameters for the assessment of drinking water quality. In: Dufour, A., Snozzi, M., Koster, W., Bartram, J., Ronchi, E., Fewtrell, L. (Eds.), Microbial Safety of Drinking Water: Improving Approaches and Methods. World Health Organization OECD, p. 2003.
Pedrero, F., Albuquerque, A., Amado, L., Marecos do Monte, H., Alarcón, J., 2011. Analysis of the reclamation treatment capability of a constructed wetland for reuse. Water Pract. Technol. 6 (3).
Pfannes, K.R., Langenbach, K.M.W., Pilloni, G., Stührmann, T., Euringer, K., Lueders, T., Neu, T.R., Müller, J.A., Kästner, M., Meckenstock, R.U., 2015. Selective elimination of bacterial faecal indicators in the *Schmutzdecke* of slow sand filtration columns. Appl. Microbiol. Biotechnol. 99, 10323–10332.
Pinheiro, M.D.O., Power, M.E., Butler, B.J., Dayeh, V.R., Slawson, R., Lee, L.E.J., Lynn, D.H., Bols, N.C., 2007. Use of *Tetrahymena thermophila* to study the role of protozoa in inactivation of viruses in water. Appl. Environ. Microbiol. 73, 643–649.
Poynter, S.F.B., Slade, J.S., 1977. Removal of viruses by slow sand filtration. Prog. Water Technol. 9, 75–88.
Redder, A., Dürr, M., Daeschlein, G., Baeder-Bederski, O., Koch, C., Müller, R., Exner, M., Borneff-Lipp, M., 2010. Constructed wetlands – are they safe in reducing protozoan parasites? Int. J. Hyg. Environ. Health 213, 72–77.

Rosegrant, M.W., Ringler, C., Zhu, T., 2009. Water for agriculture: maintaining food security under growing scarcity. Annu. Rev. Environ. Resour. 34, 205–222.
Sánchez, L.D., Sánchez, A., Galvis, G., Latorre, J., 2006. Multi-Stage Filtration. IRC International Water and Sanitation Centre, Delft, The Netherlands.
Sadiq, R., Husain, T., Al-Zahrani, A.M., Sheikh, A.K., Farooq, S., 2003. Secondary effluent treatment by slow sand filters: performance and risk analysis. Water Air Soil Pollut. 143, 41–63.
Schaefer, K., Exall, K., Marsalek, J., 2004. Water reuse and recycling in Canada: a status and needs assessment. Can. Water Resour. J. 29, 195–208.
Schijven, J.F., de Bruin, H.A.M., Hassanizadeh, S.M., de Roda Husman, A.M., 2003. Bacteriophages and *Clostridium* spores as indicator organisms for removal of pathogens by passage through saturated dune sand. Water Res. 37, 2186–2194.
Schijven, J.F., van den Berg, H.H.J.L., Colin, M., Dullemont, Y., Hijnen, W.A.M., Magic-Knezev, A., Oorthuizen, W.A., Wubbels, G., 2013. A mathematical model for removal of human pathogenic viruses and bacteria by slow sand filtration under variable operational conditions. Water Res. 47, 2592–2602.
Simpson, J.M., Santo Domingo, J.W., Reasoner, D.J., 2002. Critical review – microbial source tracking: state of the science. Environ. Sci. Technol. 36, 5279–5288.
Spanish Royal Decree, 2007. Spanish Regulations for Water Reuse. Real Decreto 1620/2007, 7 December 2007. Ministerio de la Presidencia, Madrid, Spain, pp. 2007.
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.
Toscano, A., Hellio, C., Marzo, A., Milani, M., Lebret, K., Cirelli, G.L., Langergraber, G., 2013. Removal efficiency of a constructed wetland combined with ultrasound and UV devices for wastewater reuse in agriculture. Environ. Technol. 34, 2327–2336.
USEPA, 1980. EPA 625/1-80-012, Design Manual: Onsite Wastewater Treatment and Disposal Systems. U.S. Environmental Protection Agency, Washington, D.C., USA.
US EPA, 2004. EPA/625/R-04/108, Guidelines for Water Reuse. U.S. Environmental Protection Agency, U.S. Agency for International Development, Washington, D.C., USA.
Vymazal, J., 2005. Removal of enteric bacteria in constructed wetlands with emergent macrophytes: a review. J. Environ. Sci. Health 40, 1355–1367.
WHO, 1989. Guidelines for the use of wastewater and excreta. In: Agriculture and Aquaculture: Measures for Public Health Protection. World Health Organization, Geneva, Switzerland.
WHO, 2006. Guidelines for the safe use of wastewater, excreta and greywater. Wastewater Use in Agriculture, Vol. 2. World Health Organization, Geneva, Switzerland.
Wand, H., Vacca, G., Kuschk, P., Krüger, M., Kästner, M., 2007. Removal of bacteria by filtration in planted and non-planted sand columns. Water Res. 41, 159–167.
Wang, D., Farnleitner, A.H., Field, K.G., Green, H.C., Shanks, O.C., Boehm, A.B., 2013. *Enterococcus* and *Escherichia coli* fecal source apportionment with microbial source tracking genetic markers – is it feasible? Water Res. 47, 6849–6861.
Weber-Shirk, M.L., Dick, R.I., 1997. Biological mechanisms in slow sand filters. J. AWWA 89 (1), 72–83.
Weber-Shirk, M.L., Dick, R.I., 1999. Bacterivory by a chrysophyte in slow sand filters. Water Res. 33, 631–638.
Wu, S., Carvalho, P.N., Müller, J.A., Manoj, V.R., Dong, R., 2016. Sanitation in constructed wetlands: a review on the removal of fecal indicator bacteria. Sci. Total Environ. 541, 8–22.
Yates, M.V., 2007. Classical indicators in the 21st century – far and beyond the coliform. Water Environ. Res. 79, 279–286.
Young-Rojanschi, C., Madramootoo, C., 2014. Intermittent versus continuous operation of bio sand filters. Water Res. 49, 1–10.