Water Research 267 (2024) 122429

Contents lists available at ScienceDirect

Water Research

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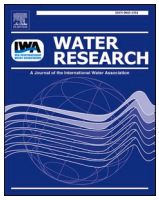

Assuring reclaimed water quality using a multi-barrier treatment train according to the new EU non-potable water reuse regulation

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ARTICLE INFO

Keywords:
EU reuse regulation
Non-potable water reuse
Virus reduction
Ceramic ultrafiltration membrane
Water reclamation

ABSTRACT

In this study, we evaluated the ability of various pilot-scale treatment train combinations to meet the microbial requirements of the new European non-potable water reuse regulation 2020/741. The study utilized non-disinfected secondary effluent from the wastewater treatment plant in Schweinfurt, Germany, as feedwater for two pilot-scale treatment trains. The first, a reference treatment train (Train A), consisted of filtration and UV disinfection as specified for reclaimed water class A in the EU regulation. The second, an advanced treatment train (Train B), included ceramic ultrafiltration (UF), ozonation, biological activated carbon filtration (BAC), and final UV disinfection. Based on a Monte Carlo simulation for Train A, the EU requirements for pathogen removal were not met when an average UV dose of 400-600 J m^{-2} was applied. This shortcoming was likely due to a moderate transmittance range (50–65 %), resulting in decreased UV fluence. These findings suggest that operational conditions for disinfection should be more clearly specified to ensure consistent pathogen inactivation both during validation and regular operation. In contrast, treatment train B successfully met the requirements of the EU regulations by reducing pathogens to below the detection limit. The UF membrane demonstrated a positive effect on the overall log reduction values (LRVs) throughout the water reclamation system. It also enhanced the efficiency of downstream processes, such as ozonation and UV disinfection, by lowering total suspended solids and turbidity. However, even without the UF membrane, treatment train B was still able to meet the pathogenic EU requirements for non-potable reuse applications. Furthermore, the study observed that the inclusion of biologically activated carbon (BAC) filtration requires a final disinfection step (e.g., UV disinfection) to prevent the potential occurrence of heterotrophic bacteria that proliferate in the BAC filter. For process validation it is recommended to use at least two different virus surrogates (MS2 and PhiX174), rather than just one or total coliphage as required in the EU regulation.

1. Introduction

Fresh water supplies are becoming increasingly scarce in many parts of the world due to urbanization, population growth, and economic development (The Global Commission on the Economics of Water 2023). The availability of water resources will become even more critical due to impacts from climate change, as the frequency of extended droughts is becoming more prevalent even in regions with moderate climate (IPCC 2022). To tackle water scarcity in arid and semi-arid regions, water reuse of municipal wastewater effluents is a promising option to provide an alternative freshwater supply (Bauer et al., 2020). Within the UN's sustainable development goals (SDG), goal 6.3 encourages to significantly increase water recycling and safe reuse globally by 2030. The 2023 World Water Development Report further emphasized the significance of non-potable water reclamation practices as alternative water resource (WWAP 2017, United Nations 2015, Helmecke et al., 2020; Ahuja, 2023).

To assure hygienically safe and environmentally responsible reuse practices, removal requirements need to be met reliably in particular for pathogens (i.e., bacteria, protozoa, and viruses) that can pose a significant and acute health risk. As a consequence, water quality standards for various types of non-potable reuse practices have been proposed and

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https://doi.org/10.1016/j.watres.2024.122429
Received 3 June 2024; Received in revised form 7 September 2024; Accepted 9 September 2024
Available online 10 September 2024

specified by several countries and international organizations (Nancy Stoner and Kadeli, 2012; Mujeriego and Hultquist, 2011; EU Parliament and the Council, 2020; Prado et al., 2019). Building upon this international experience of practice and to foster growth of reuse in Europe, the European Union (EU) introduced in 2020 for the first time non-potable water reuse regulations for agricultural irrigation, which took effect on June 25, 2023 in EU member states (EU Parliament and the Council, 2020). The EU water reuse regulation 2020/741 specifies regular monitoring requirements for the fecal indicator bacteria (FIB) *E. coli* for four water quality classes A, B, C, and D. The highest quality, class A (secondary treated wastewater followed by filtration and disinfection), includes all food crops consumed raw where the edible part is in direct contact with reclaimed water and root crops consumed raw (Table S1, supplemental information). In order to provide additional assurance in particular for class A applications, the EU reuse regulation imposed additional validation criteria to demonstrate specific minimum log removal values (LRVs) for human viruses ($\geq$ 6), bacteria ($\geq$ 5), and spores (as surrogate of protozoa) ($\geq$ 5) (Table S1). These LRVs representing the overall achieved reduction from raw sewage to finished treated water need to be validated prior to start-up of a reclamation facility or after major modifications. Both, regular monitoring for indicator organism removal ($\leq$ 10/100 mL *E. coli*) and validation requirements of LRVs shall be met at least in 90 % of all samples. California's Title 22 regulations for non-potable reuse implemented a different targeting approach, stipulating that the median concentration of total coliform bacteria in disinfected tertiary recycled water must not exceed an MPN (Most Probable Number) of 2.2 per 100 milliliters. This target can be achieved either by having 5 mg Cl_2 L^{-1} total chlorine residual after 90 min contact or demonstrate 99.999 % removal of MS2 bacteriophage at the effluent of treatment system [(California Environmental Protection Agency (CA EPA) 2018; MacDonald et al., 2024)]. Regarding to EU regulations 2020/741, a disinfectant (e.g. chlorine) residual in non-potable water reuse systems is not required.

Since detection of human viruses is often challenging due to their low abundance in treated wastewater, bacteriophages have been proposed as surrogates for treatment validation since they exhibit a similar behavior of human viruses during water treatment and established analytical protocols for their detection exist (Amarasiri et al., 2017). In a comprehensive literature review study, Heffron et al. (2024) suggested that there is no single bacteriophage serving as a conservative surrogate of human viruses for process validation. To address different virus characteristics during the validation of multibarrier treatment trains, this study selected an RNA phage (MS2) and a DNA phage (PhiX174) to represent human enteric RNA and DNA viruses, respectively. These non-enveloped phages were chosen for their similar structural properties, ease of detection, and non-pathogenic nature.

In addition, spore-forming sulfate-reducing bacteria and the spores of *C. perfringens* that are highly resistant to environmental stress and water treatment processes were proposed in the EU reuse regulation as surrogates of protozoa (Table S1). However, whether performing a one-time initial validation for the specified conditions is sufficient to assure robust removal during ongoing regular operation of a water reclamation facility can be questioned.

A well-established barrier against pathogens is UV irradiation, which can result in damaging the DNA structure of bacteria, protozoa, and viruses when appropriate operating conditions are fulfilled (Hull et al., 2019). Robustness and reliability of a treatment train could be increased by additional treatment processes. Membrane filtration like ultrafiltration (UF) can provide reliable retention of most pathogen due to the physical-mechanical separation process (Yang et al., 2021). For instance, by employing UF membranes 5 and > 6.7 LRVs of MS2 bacteriophage and *E. coli* were reported, respectively (Hull et al., 2019, Yang et al., 2021). Ozonation is another common process for pathogen inactivation, since ozone damages the bacteria's cell wall by protoplasmic oxidation, which causes cell lysis (Lee et al., 2017, Gomes et al., 2018, 2019). Commonly, ozone processes combine biological post-treatment such as biologically activated carbon filters (BAC) for removing the oxidation by-products that might form during oxidation (Bourgin et al., 2018). Advanced treatment processes like ozone and BAC also provide barriers against trace organic chemicals (TOrCs).

Given the wide range of potentially relevant pathogens and operational conditions to assure a robust and efficient removal, this study investigated how reliably a reference treatment train (comprised of secondary treatment – filtration – UV irradiation) and an advanced treatment train (secondary treatment – ceramic ultrafiltration – ozonation – BAC filtration – UV irradiation) can meet the minimal treatment requirements of the new EU reuse regulation for various non-potable reuse applications during both the required process validation and long-term operation. This study was conducted at a demonstration-scale water reuse facility located at the wastewater treatment plant (WWTP) Schweinfurt, Germany and employed a comprehensive monitoring program for pathogens to assess the risk of insufficient pathogen removal.

2. Materials and methods

2.1. The demonstration-scale water reclamation facility at the WWTP Schweinfurt

The WWTP of the city of Schweinfurt, Germany has an annual treatment capacity of 9.6 million m^3 of wastewater. The treatment train comprises several operational steps: a coarse screen, a sand and grease trap, a primary clarifier followed by biological nutrient removal using conventional activated sludge (CAS) and secondary clarification prior to discharge to the river Main. In order to explore alternative freshwater supply options in a region characterized by increasing water scarcity, a demonstration-scale water reclamation facility for agricultural and urban landscape irrigation and groundwater recharge has been established. The facility consists of two parallel treatment trains and the conceptual layout is illustrated in Fig. 1. Train A was comprised of two cartridge filters in series with a pore size of 100 and 10 μm, respectively and a UV disinfection unit (Wedeco Aquada-Series) with a flowrate of 0.5–0.7 m^3 h^{-1}. The advanced train B had a flowrate of 6 m^3 h^{-1} and was comprised of two parallel ceramic UF membranes (Table S2) with surface area of 24.7 m^2 each (Nanostone), ozonation (Xylem), biological activated carbon (BAC) filtration (two filters in series), and UV disinfection (Wedeco Aquada-Series). A bypass of reclaimed water after UV disinfection was directed to a sand column (SC), which was employed to assess the water quality variations in case of groundwater recharge (Figs. S5–S8). An additional ozonation system (Xylem) was shortly in operation prior to UF to assess the effects of pre-ozonation on coagulation and pathogen removal during microbial challenge tests. The pre-ozonation was not operated continuously as part of the treatment train B. Further details of individual components of the system are given below and in the Supplemental Information (SI). The UF operation was carried out with a pre-coagulation step using poly aluminum chloride (PACl, 10 mg L^{-1} Al^{3+}).

2.2. Sampling for microbiological analysis

Sampling for microbiological parameters occurred at multiple locations including raw sewage at the influent of the WWTP, secondary treated effluent, as well as the effluents after UF, ozonation, BAC filtration, UV irradiation, and sand column. A volume of 2 L of each sample was collected monthly in sterilized polypropylene bottles during multiple campaigns between September 2021 and February 2024.

2.3. Microbial assays

The primers used in this study for quantifying bacteria and viruses are described in Table S4. In short, the applied culture-based methods followed standardized procedures and are summarized in Table 1.

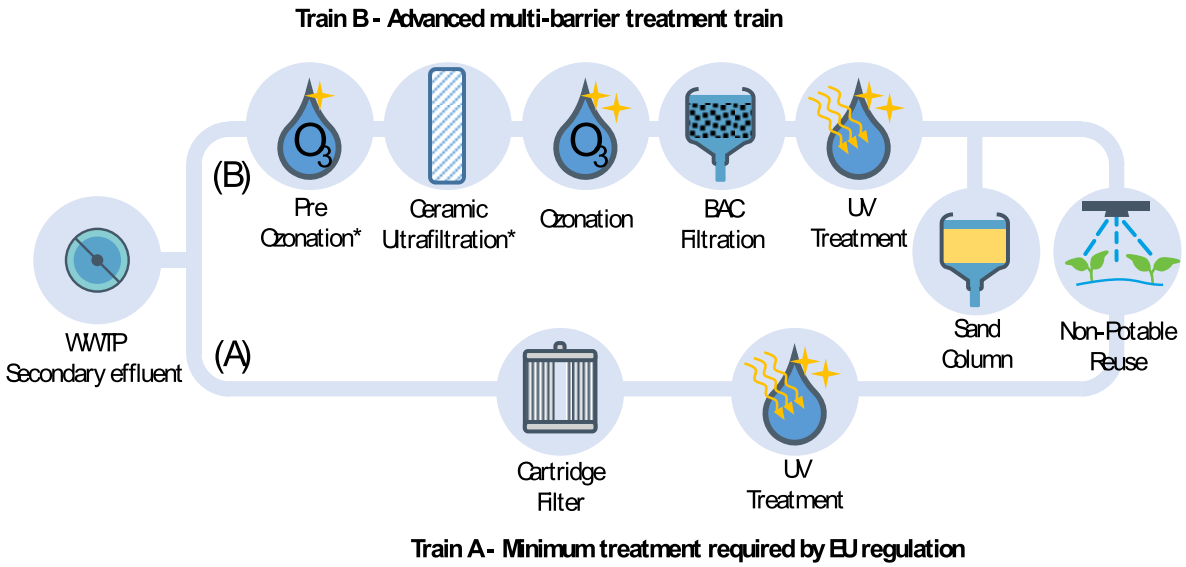

Fig. 1. Conceptual layout of the two parallel multi-barrier treatment trains (A and B) at the Schweinfurt water reclamation facility. * Train B was operated with and without pre-ozonation and ultrafiltration.

Table 1
Overview of methods for quantifying bacteria, viruses and resistance genes, with all parameters measured in their respective units such as MPN (Most Probable Number) or CFU (Colony Forming Units) for bacteria, PFU (Plaque Forming Units) for viruses, and gene copies for resistance genes.

Parameter	Method	Unit
E. coli	DIN EN ISO 9308-2:2014	MPN
Coliforms	DIN EN ISO 9308-2:2014 Bacterial identification with MALDI-TOF-MS	MPN
Pseudomonas aeruginosa	DIN EN ISO 16266	PFU
Enterococci	DIN EN ISO 7899 Bacterial identification with MALDI-TOF-MS	CFU
Most probable number (MPN) of cultivable bacteria	R2A medium, room temperature, 7 days	MPN
Clostridium perfringens spores	DIN EN ISO 14189:2016 with pre-heating samples at 60 °C for 10 min	CFU
Somatic coliphages (incl. PhiX174 for spiking tests)	DIN EN ISO 10705-2:2002	PFU
F-specific coliphages (incl. MS2 for spiking tests)	DIN EN ISO 10705-1:2002	PFU
Intact cell count (ICC)	Flow cytometry (SG + PI)	ICC
Bacterial regrowth (of all bacteria)	ICC of day 0 and after 7 days of storage at room temperature	Regrowth factor
Human pathogenic viruses	Digital droplet PCR (see 2.5.5)	genetic copies
Plant viruses	Digital droplet PCR (see 2.5.5)	genetic copies

2.4. Microbiological spiking test for individual process validation

Since the abundance of some human pathogens, in particular viruses, in raw sewage, secondary effluent or after advanced treatment like ultrafiltration was too low to demonstrate removal of the required LRVs according to the EU reuse regulation, spiking tests using two different surrogates of human enteric viruses were carried out: F-specific MS2 phages (RNA virus) and somatic PhiX174 phages (DNA virus) (Hambsch et al., 2012). Viral stock solutions were produced and quantified according to standardized methods (DIN-ISO 10705-2, DIN-ISO 10705-1). The concentrations of MS2 and PhiX174 phage in the stock solution were about 10^{11} phages per mL.

The spiking of phages was conducted for each treatment process of train B separately. For each treatment process, a 60 l baffled tank containing tap water spiked with both phages at a final concentration of 10^7 phages per mL was prepared and mixed gently for 1 h using a sterilized overhead stirrer. The phage mix was pumped into the main feedline prior to a static mixer using a progressive cavity pump with a flow rate of 25 L h^{-1}. The flow rate of the pilot plant during these experiments was 2 m^3 h^{-1}. Assuming proper mixing of phages in the main feedline, the theoretical concentration of both phages in the inflow of each process was approximately 10^5 per mL (Fig. 2). Various key operational set-points (i.e., membrane flux; specific ozone dosages; UV fluence) were adjusted during dedicated experiments as summarized in Table 2.

During the spiking experiments, we collected samples from the influent and effluent lines of each treatment process to evaluate removal efficiencies. To determine the initial concentration of phages and account for any interference from natural phages, we also sampled the influent and effluent before the spiking period. Additionally, samples were taken directly from the spiking tank to verify the applied dose of phages in the inflow line. Each sample was a composite of three individual samples collected during the experiment.

The BAC filters were sampled more frequently to monitor the retention and desorption behavior of both phages through the filter. All samples were stored at -20 °C pending further analysis. In order to reduce cross-contamination during subsequent experiments, spiking tests were carried out from the downstream to the upstream processes of the pilot plant (spiking started prior to UV, followed by BAC filtration, ozonation, and finally UF). Spiking experiments were performed for the advanced treatment train B once during a period without the UF membrane in operation and once with the UF membrane to assess the impact of UF on downstream processes.

2.5. Data analysis, statistics and visualization

All LRVs were calculated using a Monte Carlo simulation. In each loop, separate random sample sets for the inflow and outflow of the observed treatment (e.g. inflow / outflow UV) were generated. From the two sample sets, single LRVs were calculated for each sample pair. From all calculated LRVs, a Kernel Distribution Estimation (KDE) was generated (kde method of the scipy library in Python). The final represented LRV is the 10th percentile of this KDE (Fig. 3). For results falling below the LOD, the LOD value itself was utilized to calculate the LRV,

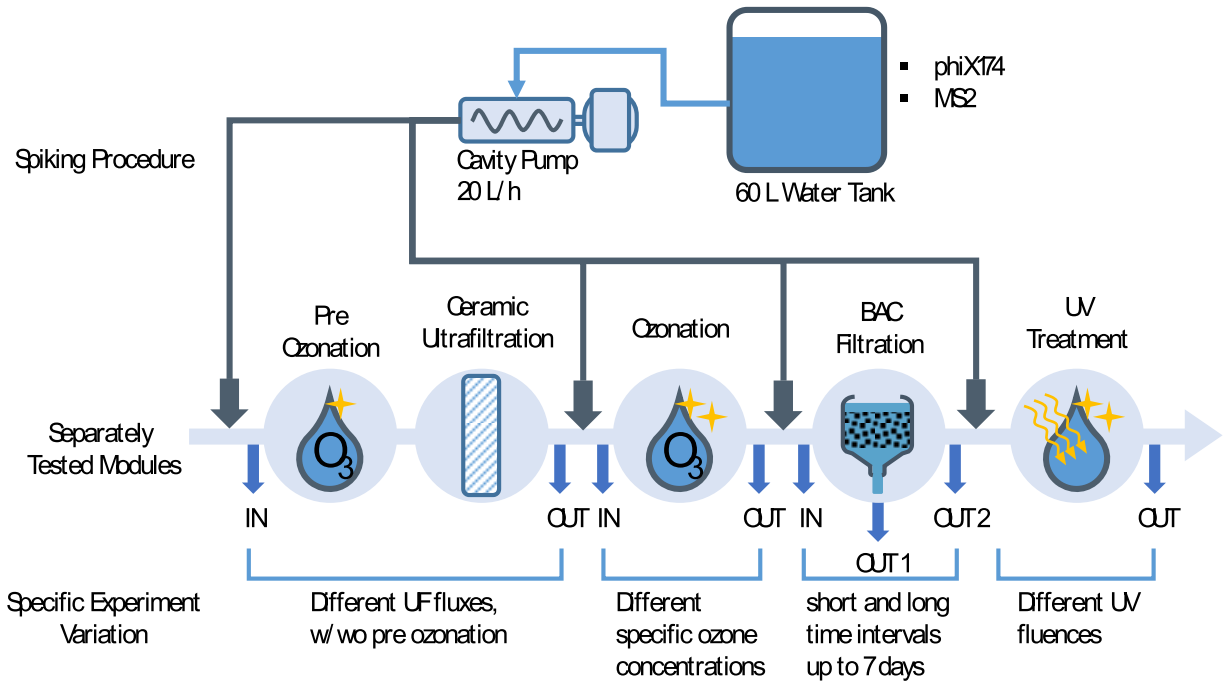

Fig. 2. Experiment scheme of the microbial spiking tests with MS2 and PhiX174 phages. Blue arrows indicate sampling locations.

Table 2
Operation set-points tested during the spiking tests with MS2 and PhiX174 phages.

Treatment unit	Operation set-point	HRT	Pretreatment unit(s)
Pre-ozonation	0.2 g O_3 (g DOC)$^{-1}$	20 min	-
UF	100 LMH	11 min	*with and without pre-ozonation*
	120 LMH	11 min	
	150 LMH	11 min	
	180 LMH	11 min	
Ozonation	0.2 g O_3 (g DOC)$^{-1}$	25 min	*with and without UF*
	0.4 g O_3 (g DOC)$^{-1}$	25 min	
	0.6 g O_3 (g DOC)$^{-1}$	25 min	
	0.8 g O_3 (g DOC)$^{-1}$	25 min	
	1.0 g O_3 (g DOC)$^{-1}$	25 min	
BAC filtration	-	30 min	*with and without UF and main ozonation*
UV fluence	370 J m^{-2}	-	*with and without UF, main ozonation, BAC filtration*
	430 J m^{-2}	-	
	500 J m^{-2}	-	
	600 J m^{-2}	-	
	700 J m^{-2}	-	
	900 J m^{-2}	-	
	1,400 J m^{-2}	-	
	1,850 J m^{-2}	-	
	2,300 J m^{-2}	-	

employing a conservative approach. In the spiking experiments, sample sets represented replicates of inflow and outflow concentration measurements for a single treatment step under specific conditions (e.g., ozone at 2 mg L^{-1}). For routine monitoring, unpaired sample sets were used for calculations due to the unequal number of samples in some pairs. Using only paired samples would have led to a loss of data for certain treatment steps.

All data analyses were performed in Python (3.11.5) with the pandas (2.0.3), numpy (1.24.3) matplotlib (3.7.2), and seaborn (0.12.2) modules. FCS (Flow Cytometry Standard) data was analyzed using shapely (2.0.4) and fcsparser (0.2.8). Figures were created in Python with the seaborn module.

3. Results and discussion

In order to assess how reliably the requirement of the new EU non-potable reuse regulation can be met for microbial parameters, a reference treatment train A (sec. effluent – filtration – UV) and an advanced treatment train B (sec. effluent – UF – ozone – BAC – UV) were compared in parallel while adjusting various operational set-points at a demonstration-scale water reclamation facility.

3.1. The abundance of target pathogens in raw sewage and non-disinfected secondary effluent

In this study, we executed a comprehensive microbial screening program for various bacteria, *Clostridium perfringens* spores, and viruses relevant to the EU reuse regulation requirements. Abundances of these targets in the raw sewage and secondary effluent are comparable with literature and illustrated in Fig. S1 (Ajonina et al., 2015; Antony et al., 2014).

The total number of intact and cultivable bacteria in the WWTP inflow was around 10^9 cells per 100 mL, thus reflecting a significant portion of the bacterial population (Mohr et al., 2020).

The abundance of human pathogenic viruses can be highly variable depending on processes, operating conditions of a WWTP, and the shedding behavior within the serviced community. Concentrations in raw sewage can vary from 10^1 to 10^8 genomic copies per 100 mL for human adenoviruses, norovirus GII, enteroviruses, and papillomaviruses (Sommer et al., 1998; Malayeri et al., 2006; Hijnen et al., 2006, U.S. EPA, 2020; Pirnie et al., 2006,McCall et al., 2020) In this study, the nucleic acids of adenoviruses, enteroviruses, rotaviruses, polyomaviruses, SARS-CoV-2 as well as PMMoV, TMV and CrAss-phage

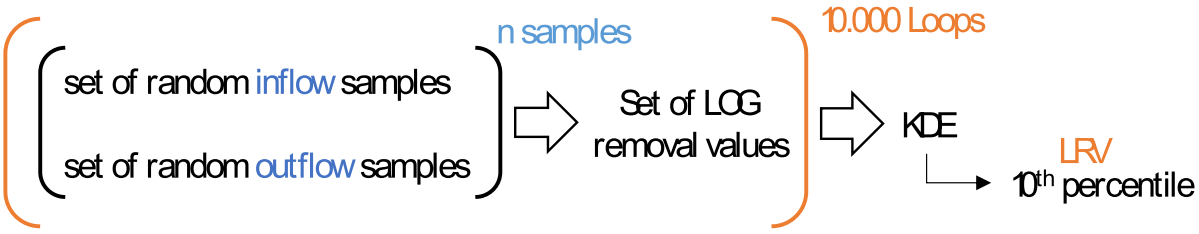

Fig. 3. The procedure of calculating LRVs using kernel density estimation (KDE).

could be detected in raw sewage. Compared to bacterial indicators, the abundances exhibited higher variation and beside the indicator viruses PMMoV, TMV and CrAss-phage, all viruses were lower concentrated than F+ and somatic coliphages. Due to this observation, our study focused on F+ and somatic coliphages. Our measurements of human pathogenic viruses may be used for quantitative microbial risk assessment (QMRA) in future studies. All raw data of acquired concentrations and analysis of this study can be found in Table S3.

3.2. *Performance of the reference water reclamation train – Train A*

The removal of indicator organisms including *E. coli*, total coliform, and *C. perfringens* spores through the treatment by CAS followed by filtration and UV disinfection has been determined and is illustrated in Fig. 4. Although this treatment train with two cartridge filters in series and an average UV fluence of approximately 490 J m^{-2} (see SI-4 for details) can meet the performance target for bacteria ($\geq$ 5 log for *E. coli*), it did not meet the FIB requirement for reclaimed water quality (*E. coli* <

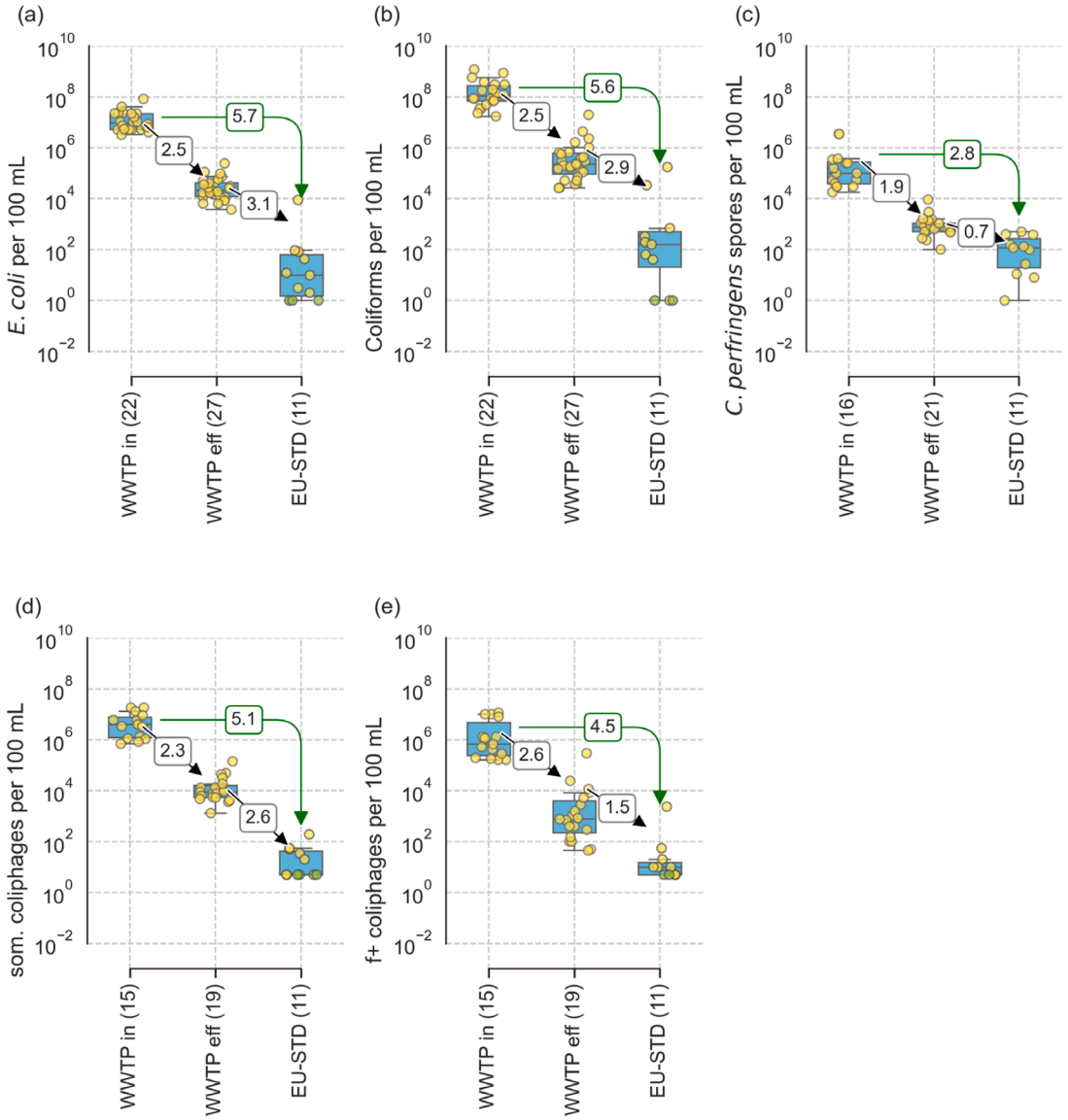

Fig. 4. Pathogens removal of train A applying conventional secondary treatment, filtration, and UV disinfection (UV dose ≈ 490 J m^{-2}). Arrows demonstrate calculated LRVs with the 10th percentile value indicated (90 % of the values are greater than the indicated value). The green arrow shows the total log removal. Numbers in brackets represent n.

10 per 100 mL) in 90 % of all samples (Section 3.4). The performance targets for validation for protozoa (≥ 4 log for *C. perfringens* spores) and viruses (≥ 6 log for coliphages) were only partially met (Fig. 4). While all indicator organisms were removed by 1.9 to 2.6 log during conventional wastewater treatment, inactivation by downstream processes was less effective for spores and coliphages. All target organisms were frequently detected in the reclaimed water. It should be noted that the small-scale UV reactor used in this study has been operated at constant UVC output without adjusting UV dose to variable UV transmittance (Fig. S11). Applied UV doses of 430 J m^{-2}, 490 J m^{-2} and 620 J m^{-2} were determined for minimum, average and maximum measured UV transmittance, respectively, using the point source summation (PSS) method. Observed UV disinfection efficiency followed expected trends based on established UV sensitivities for indicator organisms with *E. coli* ≈ somatic coliphages > F+ coliphages > spores of *C. perfringens* (Antony et al., 2014, Ferrer et al., 2015), but due to the low average UVT (60 %) the applied UV doses were too low to reliably meet the EU minimum requirements for reclaimed water quality and treatment performance.

In order to reliably meet the performance targets of the EU regulation, UV fluences need to be adjusted according to well reported UV sensitivities of indicator organisms (Carabias et al., 2023). For instance, assuming ≈ 2 LRVs of indicator organisms by CAS, the required UV fluences for final UV disinfection have to be in the range of < 200 J m^{-2} for ≥ 3 log *E. coli*, ≈ 900 J m^{-2} for ≥ 4 log coliphages (mean value from 262 dose-response curves in US-EPA (2020)), and ≈ 660 J m^{-2} for ≥ 2 log *C. perfringens* spores (Antony et al., 2014; Khan and Anderson, 2018; Leister and Hügler, 2022; Rizzo et al., 2020). This can be accommodated by the use of validated UV reactors providing reduction equivalent doses (RED) as described in established validation protocols (Pirnie et al., 2006). Since average UV doses determined using point source summation can substantially differ from RED for individual organisms, directly comparing the pathogen removal with reported values from the literature remains challenging. Depending on the conservatism of other assumptions, an additional safety factor such as 95th percentile bounds might be needed to achieve performance targets exceeding 90 % in all samples.

A few reclaimed water samples showed exceptionally high concentrations for different indicators, e.g. *E. coli* was detected at concentration up to 10^4 per 100 mL Monitoring turbidity levels after the cartridge filters indicated sporadic improper performance with elevated turbidity levels (Fig. S11), which might have caused limited disinfection efficiency at times. Consequently, full-scale treatment facilities for water reuse should be equipped beside online monitoring of turbidity (as required by EU regulation), with online monitoring of UV transmittance and UV intensity to indicate failures during operation of filters or UV disinfection.

3.3. Performance of the advanced water reclamation train (Train B)

The pathogens removal of the advanced treatment train (train B) was initially operated over six months without UF followed by 22 months operation with UF. Removal performance was assessed during both periods.

3.3.1. Removal of fecal indicators

The removal of fecal indicators, including E. *coli*, total coliforms, and *C. perfringens* spores, in train B with and without UF resulted in similar log reduction values (LRVs) of ≥ 7.0 / ≥ 6.8 for *E. coli*, ≥ 7.8 / ≥ 7.9 for total coliforms, and 5.0 / ≥ 4.7 for *C. perfringens* spores, respectively. The concentrations of FIB were mostly below the LOD in both operation scenarios (Fig. 5). *E. coli* concentrations in the reclaimed water of train B with and without UF membrane always met the EU requirements for non-potable reuse applications (class A, ≤10 per 100 mL, in 90 % of all samples).

During operation without UF, ozonation with a specific ozone dosage of 0.6 g O_3 (g DOC)$^{-1}$ exhibited an average *E. coli* removal of 3.2 LRVs. Lüddeke et al. (2015) reported only 1-2 LRVs for *E. coli* applying a specific dose of 0.73 mg O_3 (mg DOC)$^{-1}$. This different removal efficiency might be due to differences in ozone demand, type of ozone injection, reactor design, or TSS concentrations in secondary effluent. This underscores the importance to conduct performance validation for each reclamation facility to account for site specific conditions. Thus, literature data can only provide an estimation of expected removal performance and is less applicable when deviating operating conditions are established.

During long-term operation, a slight increase of *E. coli* concentrations was observed after the BAC filter. This effect might have been caused by bacterial regrowth as reported by Reaume et al. (2015) (Reaume et al., 2015). However, the applied UV irradiation with an average UV dose of ≥550 J m^{-2} (determined at max. flow of 1500 L h^{-1} and min. measured UVT of 81 %) reliably reduced any remaining *E. coli* below LOD. With the ceramic UF membrane in operation, no *E. coli* could be detected after UF. Consequently, the log removal of the UF determined as >1.3 LRVs was likely underestimated. Since the concentration in the UF effluent was close or below the LOD, LRVs for downstream processes could not be determined. Although, the UF serves as a highly efficient pathogen barrier, any integrity issues could comprise virus removal in particular. Conventional integrity testing of UF membrane using pressure holding tests can overestimate membrane performance regarding virus rejection. Thus, different approaches have been proposed such as the use of a non-microbial indicator with a net negative surface charge (citrate stabilized silver nanoparticles) to assess membrane integrity (Antony et al., 2014). However, regular microbial challenge tests using bacteriophages are another possibility to assess membrane integrity (Ferrer et al., 2015). Furthermore, for a comprehensive microbial risk assessment, knowledge regarding the efficiency of downstream processes is needed (Khan and Anderson, 2018). This insight could be gained by spiking indicator organisms, adopting literature data, or performing process validation.

During the period without employing the UF membrane, the ozonation process resulted in average removal of 2.6 LRVs for coliform bacteria. However, after adding the UF to the treatment train, regrowth of coliforms (2.7 log) after ozonation occurred, although the UF already reduced the coliforms to below LOD (≥ 5.2 log reduction) (Fig. 5 d). Gerrity et al. (2011) also detected coliform regrowth in the last stage of their advanced treatment system (CAS+UF+O_3/H_2O_2+BAC) and suggested a final disinfection to mitigate the issue. In order to determine the type and source of this contamination in the ozonation system, the coliform bacteria were identified with MALDI-TOF-MS. A very low biodiversity of coliform bacteria was observed after ozonation and BAC filtration (Shannon-Wiener-Index of ≤ 0.1 compared to 0.9 in secondary effluent). Overall, 79 % of species were identified as *Lelliottia amnigena*, 7 % as *Serratia fonticola*, 4 % as *Citrobacter freundii*, 3 % as *Enterobacter taylorae,* and 7 % as other species (Fig. S9). While these bacteria are not typically associated with health risks, their presence suggests internal contamination. In general, the percentage of *Lelliottia amnigena* increased from the first sampling campaign from about 17 % to nearly 100 % in the following months of monitoring. *Lellottia amnigena* was reported to grow in oligotrophic environments like drinking water reservoirs (Leister and Hügler, 2022). Regrowth with a single bacterial species as well as the absence of *E. coli* indicated an internal contamination of the treatment system. It should be mentioned that before operating the UF prior to the ozonation system, once in a while elevated levels of total suspended solids (4–8 mg L^{-1}) were detected in the secondary effluent feeding the ozonation system resulted in clogging of sampling ports, which might have caused coliform contamination (Fig. S9). This issue was resolved by disinfecting the ozonation system using sodium hypochlorite, resulting in full removal of coliforms after ozonation and low amounts of *Lelliottia amnigena* in the BAC filter effluent (3 to < 1 coliforms per 100 mL). During long-term operation, the final UV irradiation (≥ 550 J m^{-2}) resulted in removal of coliform bacteria below LOD except for two positive detects (out of 19) that were caused by the growth of *Lelliottia amnigena* in the previous treatment

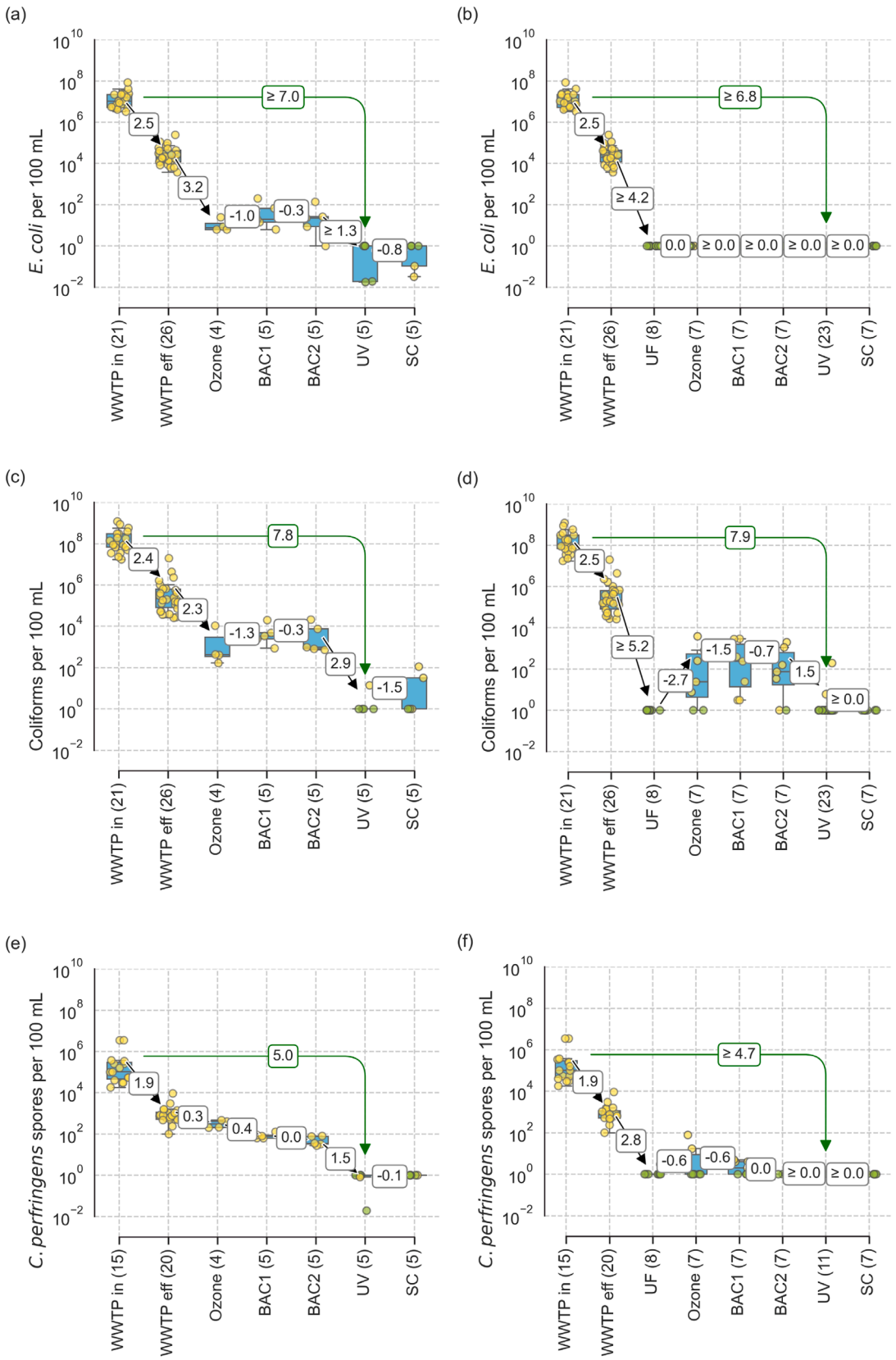

Fig. 5. Fecal indicators removal by treatment train B from raw sewage to final effluent (sec. effluent - (with or w/o) UF-ozonation-BAC-UV) during continuous operation. Graphs a, c, and e show the pathogens removal without UF and graphs b, d, and f show the pathogen removal after adding UF to train B. As expected, the sand column (SC) did not affect the pathogens removal. An ozone concentration of 0.6 g O_3 (g DOC)$^{-1}$ and an average UV dose ≥550 J m^{-2} were applied. The EBCT of BAC filters at the last sampling campaign was 25,000. Arrows demonstrate calculated LRVs with the 10th percentile value indicated (90 % of the values are greater than the indicated value). The green arrow shows the total log removal. Numbers in brackets represent n.

steps in combination with a technical failure of the UV lamp. After the one-time system disinfection, no coliforms could be detected after the UV unit.

After UF treatment, the concentration of *C. perfringens* spores reached the LOD. High bacteria and spores removal between 2-4 LRVs by UF was also reported in previous studies (Ferrer et al., 2015, Im et al., 2018). In contrast to *E. coli* and coliform bacteria, *C. perfringens* spores exhibited a higher degree of resistance during ozonation with a reduction of only 0.4 log. *C. perfringens* spores were reported as much more resistant to disinfection processes, including ozonation and UV treatment than vegetative bacteria. Lanao et al. (2008) claimed that 3.6 mg O_3 L^{-1} was necessary to achieve 4 log removal of *C. perfringens* spores in drinking water treatment. In this study, the reduction of *C. perfringens* spores by ozonation during operation without UF was significantly less pronounced (0.4 log) due to the rapid ozone decay in the wastewater matrix leading to comparably low ozone exposures. Similarly, Sauter et al. (2021) have observed 0.5 log reduction of *C. perfringens* spores after applying 0.7 g O_3 (g DOC)$^{-1}$ in secondary effluent (Sauter et al., 2021). Also for UV treatment, Hijnen et al. (2006) reported a maximum of 3 log reduction of *C. perfringens* spores employing 640 J m^{-2} (Hijnen et al., 2006). Results from Carabias et al. (2023) indicated required UV fluences of 330 J m^{-2} per log reduction. Determined LRV from this study was slightly lower with an average UV dose of ≥550 J m^{-2}, but it should be noted that measured concentration in the effluent of the UV reactor was often below LOD. The performance target of LRV ≥ 4.0 for protozoa employing the advanced water reclamation train B could be met in >90 % of all samples.

To further characterize the microbial composition of reclaimed water, intact cell counts (ICC) were analyzed using flow cytometry (BactoSense, bNovate, Switzerland). ICC results do not correlate with any other hygienically relevant microbial contaminants and their removal in the treatment system. Therefore, the ICC is not suitable as an indicator of microbial quality, but can give insights to internal processes like regrowth in the effluent of multi-barrier treatment facilities and storage tanks (Nocker et al., 2020). An average reduction of intact cell count up to 3 LRVs was observed during primary and secondary wastewater treatment (Fig. S2), about 1 LRV by ozonation (without UF as pretreatment), and 1.8 log reduction by ultrafiltration. Previous studies reported similar results (Mohr et al., 2020). During UV irradiation (≥550 J m^{-2}), no significant removal of intact cells was observed. Applying low to moderate UV fluences damages the DNA (Wigginton and Kohn, 2012), but keeps the cell membranes intact, which is an undetectable damage for ICC monitoring using flow cytometry (Van Nevel et al., 2017). To further investigate the relatively low log removal of intact cells by UF in our study (1.8 LRVs), multiple sampling ports considering the hydraulic retention time (HRT) directly prior to the UF module as well as between the UF module and permeate tanks were analyzed using online flow cytometry. The results revealed that the ICC was reduced to numbers below the detection limit directly after the UF module representing an LRV of about 4.5 (Fig. 6). Shortly after, elevated levels of ICC could be measured in the pipelines between the UF module and the permeate tanks. The increased content of high nucleic acid cells

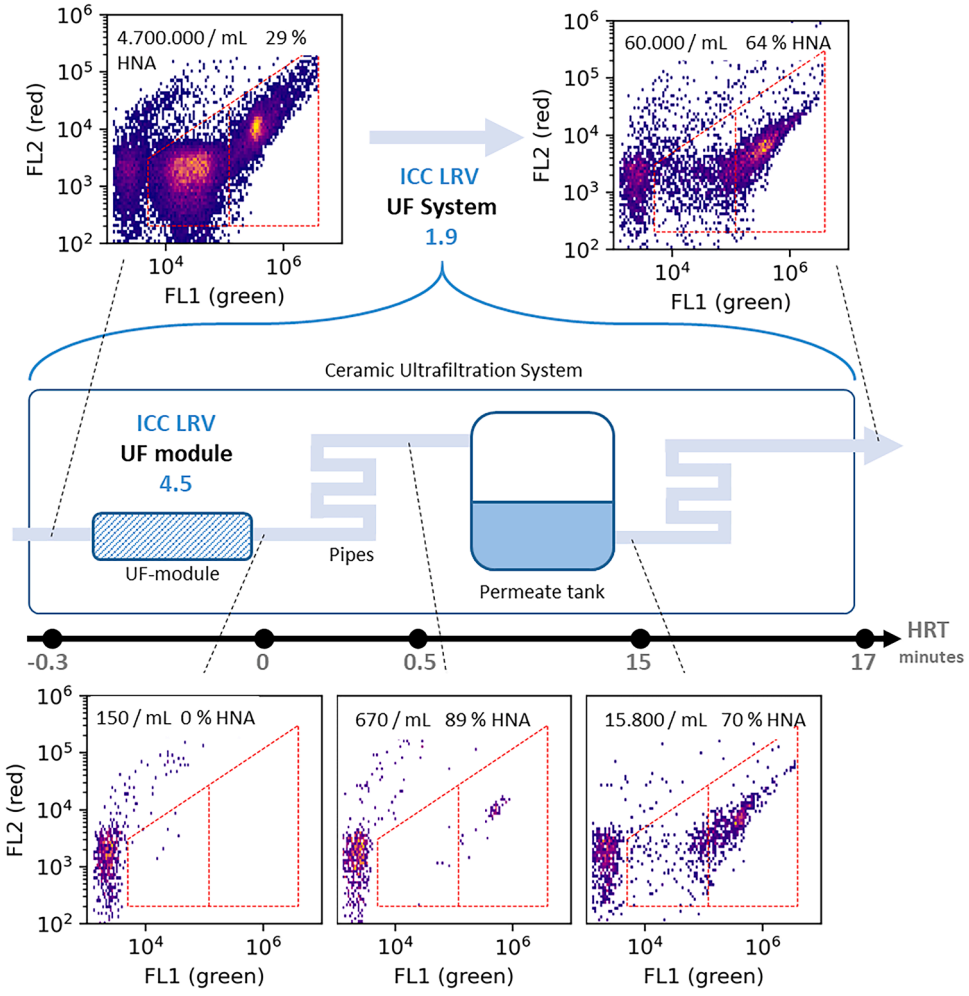

Fig. 6. Cell removal and regrowth in the UF system based on flow cytometric fingerprints. These results demonstrate a high regrowth rate in UF treated water (in the pipeline and permeate tank).

(HNA) suggests an internal regrowth (non-pathogenic) in the UF system, but also confirms the LRVs measured for bacterial indicators. This underscores the potential of flow cytometry as monitoring tool and emphasizes the importance of selecting the appropriate sampling location based on the monitoring objective. For monitoring regrowth, it is crucial to sample throughout the entire piping system, whereas to assess ultrafiltration efficiency, sampling should be done directly after ultrafiltration.

Considering the quality of the BAC filtration effluent, findings of this study reveal that the overall bacterial concentration remains within the range of 10^5 and 10^7 per 100 mL. Notably, bacteria of hygienic concern such as *E. coli* or *Enterococci* were effectively reduced. It is generally preferred to maintain a stable bacterial community as it can mitigate the risk of pathogen resurgence, making it a more favorable outcome compared to achieving sterile water by highly advanced treatment such as high-pressure membranes (Gerrity et al., 2011). This notion is supported by the measured bacterial regrowth potential, wherein all effluents post-BAC filtration displayed a regrowth factor close to 1 (indicating no significant regrowth within one week). In contrast, samples treated by UF (Fig. 6) and ozonation exhibited markedly higher regrowth factors, reaching up to 1,000-fold increase in bacterial concentration within the same timeframe. In other parts of the world such as California, it's favorable to apply residual chlorine in non-potable reuse distribution systems in order to protect the consumer and prevent the bacterial regrowth (California Environmental Protection Agency (CA EPA) 2018). Hydrogen peroxide is another effective alternative for disinfection and preventing bacterial regrowth in distribution systems (Szczuka et al., 2021).

3.3.2. *Removal of human virus surrogates*

In this study, somatic (Fig. 7 a and b) as well as F+ coliphages (Fig. 7 c and d) were evaluated as viral surrogates. The advanced treatment train B exhibited removal of $>$ 5.8 LRVs for somatic coliphages both without and with UF. For F+ coliphages, only a lower removal of $\geq$ 5 log could be determined due to the lower concentration in the WWTP influent. Similar to FIB, coliphage concentrations reached levels below

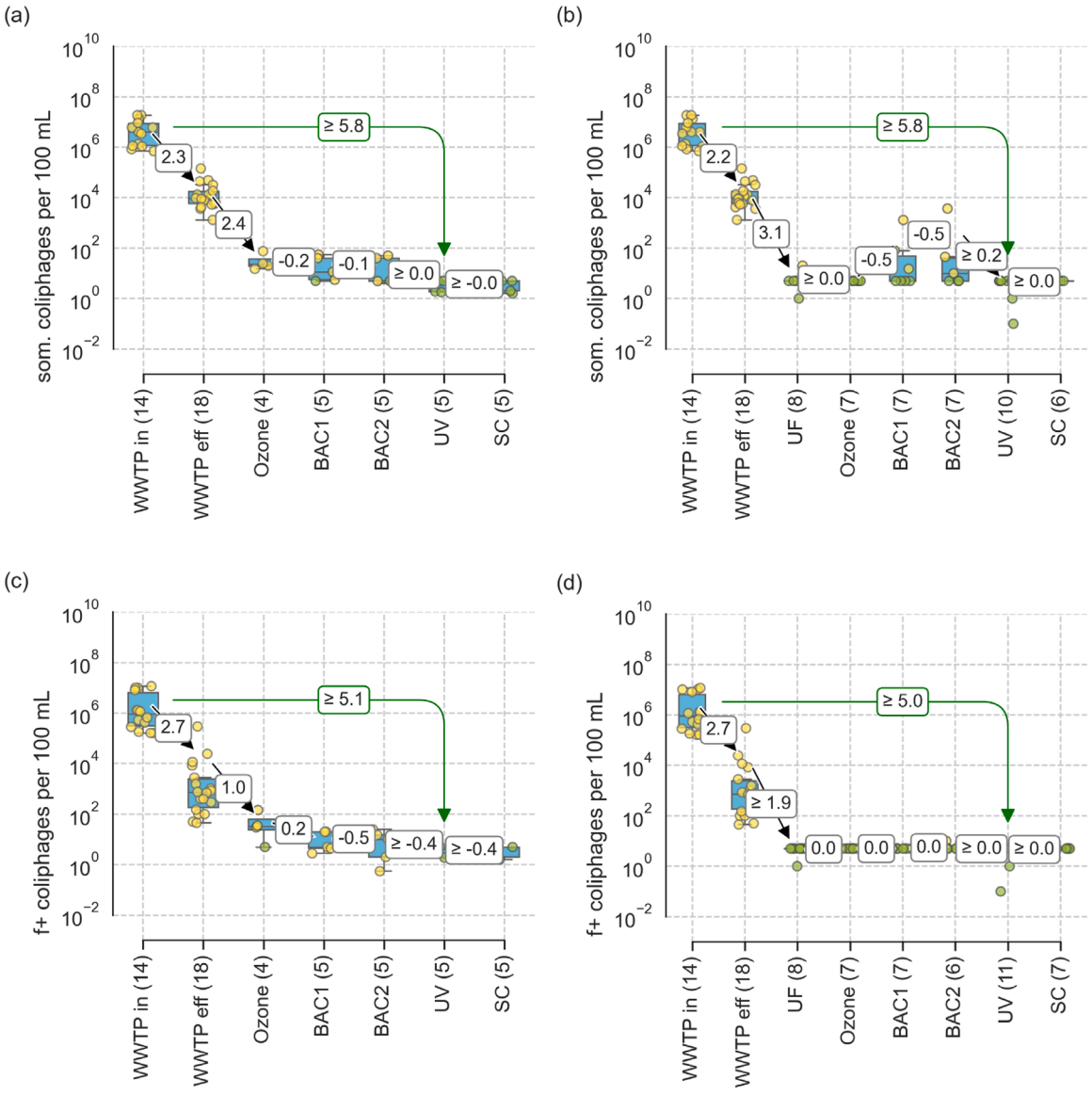

Fig. 7. F-specific and somatic coliphages removal efficiency of treatment train B from raw sewage to final effluent (number in parenthesis represents number of sampling campaigns during continuous operation). Arrows indicate calculated log removal values with the 10th percentile value indicated (90 % of the values are greater than the indicated value). The green arrow shows the total log removal (until UV treatment), the black arrows the log removal of interim treatments. Numbers in brackets represent n.

the LOD after the UV process for the period without operating the UF membrane. With UF as the first barrier of the advanced treatment train, the concentration of F+ coliphages was already below LOD in the UF filtrate. Consequently, potential log reduction credits of the downstream processes including ozonation, BAC filtration and UV irradiation could not be determined anymore. In the case of somatic phages, a few phages (around 13 per 100 mL on average) could still be measured in about half of the samples after ultrafiltration.

During operation of the UF, the observed concentrations of somatic coliphages indicated a slight increase in the BAC filters (Fig. 7 b). This was not observed for F-specific phages. The positive findings likely originate from samples taken after the spiking tests, which suggests that coliphages are washed out of the BAC filter over a longer period of time (see following section).

Based on the obtained results of regularly monitoring the performance of train B, the validation requirements of the EU regulations for bacteria (*E. coli* ≥ 5 LRVs) and *C. perfringens* spores as surrogate of protozoa (≥ 5 LRVs) measured as the 10th percentile of difference between raw sewage abundance and final reclaimed water quality were met. Exceeding the requirements for virus removal (coliphages ≥ 6 LRVs) could not be demonstrated due to reaching the detection limit resulting in lower LRVs for both somatic (≥ 5.8) and F+ coliphages (≥ 5.0). Even if the total sum of all LRVs is considered sufficient treatment according to the EU regulation, more precise information on the possible LRVs from literature (e.g. validated UV fluences) is essential for risk management.

3.4. Monte Carlo simulation of pathogen removal capacity of reclaimed water systems

In order to assess whether the performance targets (bacteria, coliphages, and *C. perfringens* spores) according to the EU reuse regulation Class A can be reliably met, probability density functions of LRVs were computed for *E. coli, C. perfringens* spores, and coliphages representing different treatment train combinations (train A: CAS-filtration-UV; train $B_{no\text{-}UF}$: CAS-ozonation-BAC-UV; train $B_{with\text{-}UF}$: CAS-UF-ozonation-BAC-UV) (Fig. 8). For train A, considering the obtained results after Monte Carlo (MC) simulation, a reliable pathogen removal for a required 90 % percentile of all sampling campaigns could not be guaranteed for all indicator microorganisms with the applied average UV dose of ≈490 J m^{-2}. The results suggest that proper operation of filtration and UV disinfection employing higher fluences are required to reliably meet performance targets for viruses and spores. The EU reuse regulation is not specifying operational conditions for the filtration or disinfection process other than setting TSS and turbidity standards. It should be noted that by increasing the applied UV fluence the acquired removal distributions in Fig. 8 can be shifted to the right (resulting in higher removal rates) while meeting the requirements of the EU reuse regulations for train A.

The MC simulation of LRVs for the advanced train $B_{no\text{-}UF}$ could achieve the 90 % percentile removal goal (> 5 log) reliably for *E. coli* and for *C. perfringens* spores (> 4 log). The removal requirement for coliphages couldn't be demonstrated since monitoring data were below the LOD. The actual removal rate was higher than measured. It should be noted that during the sampling campaigns during regular operation, all

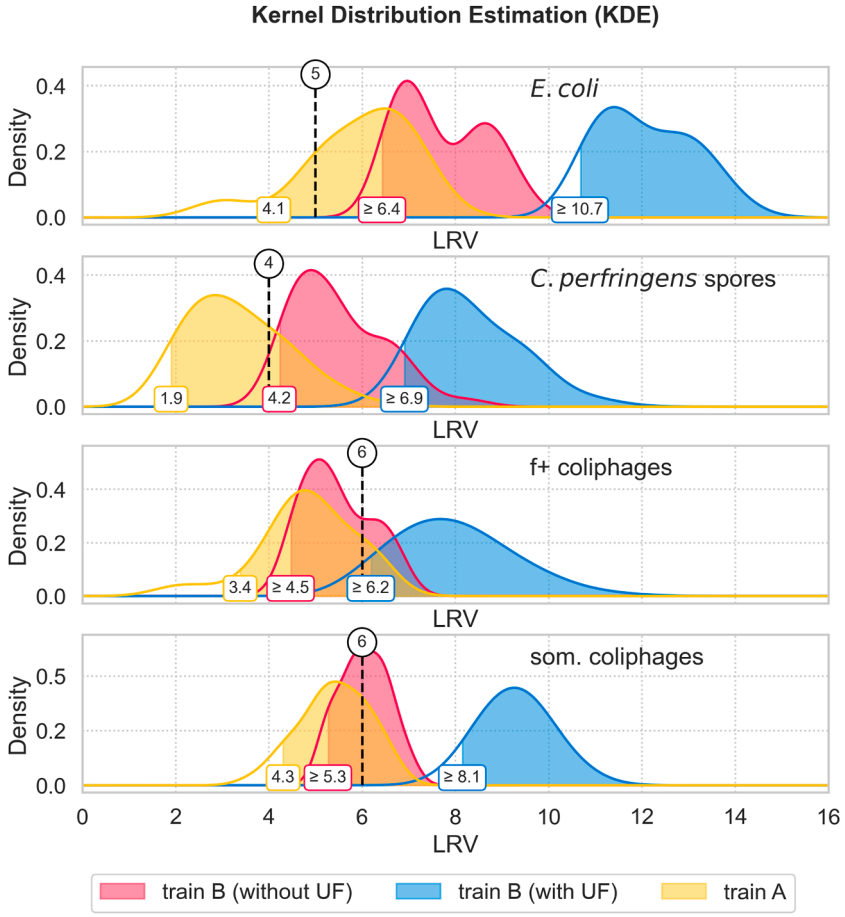

Fig. 8. The KDE of LVRs for simulated EU treatment train for minimum requirements of non-potable water reuse (CAS-filtration-UV) (orange plot) and the multibarrier treatment train (CAS-UF-ozonation-BAC-UV) (blue plot) in water reclamation facility of Schweinfurt. The 90 % percentile of each data set are illustrated. Additionally, the minimum requirements of EU commission for non-potable water reuse are indicated by black dashed line.

indicator pathogens removal requirements were met. The MC simulation signified all possible scenario combinations including the worst and best removal rates for all treatment barriers of the system. However, this is not representing operational reality and is a very conservative way of evaluating pathogen removal by a given treatment train.

The demonstration of probability density functions for train $B_{with\ -UF}$ was not possible due to the high removal after UF (below LOD). Therefore, the blue plot representing train $B_{with-UF}$ in Fig. 8 is plotted from the sum of simulated LRVs of CAS-UF and the achieved LRVs with the train B (ozone-BAC-UV) prior to UF installation. Treatment train $B_{with-UF}$ reliably met all the required standards of the EU reuse regulation for pathogen removal. The lowest simulated removal resulted from the MC simulation illustrated for F-specific coliphages with a 90 % percentile of 6.2 LRVs, which was due to their low concentrations in secondary effluent. The following section (3.5) explores the notably higher removal rates of coliphages implementing microbial challenge tests. Moreover, the f-specific coliphages including MS2 phages belong to the most conservative viral pathogen indicators in water reuse application and risk assessment processes (Wigginton and Kohn, 2012).

Due to the highly different behavior of PhiX174 and MS2 observed during both UF and UV treatment, we recommend monitoring and reporting both F+ and somatic coliphages during process validation, contrary to the EU regulations (Table SI1), which advocate for either measuring total coliphage or if that is not possible only somatic or F+ coliphages. The use of total coliphages may obscure the ability of a desired treatment train to effectively target specific pathogens.

Given the observed variations for the LRVs of pathogens in our study, a higher level of confidence could be reached by understanding the robustness of the individual treatment barriers comprising an overall treatment train. Therefore, for a more comprehensive assessment of risks, microbial challenge tests were conducted to evaluate the log removal capacity of individual processes in more depth.

3.5. *Validating pathogen removal performance of individual processes by microbial challenge tests*

3.5.1. *Determining LRVs for the ceramic ultrafiltration process*

The spiking test for the UF process of treatment train B with pre-coagulation was carried out in two phases, with and without pre-ozonation. The results of MS2 and PhiX174 phages removal during UF treatment at different fluxes to validate the reliability of UF for pathogen removal as the first stage of the multi-barrier treatment train B are shown in Fig. 9 a. The removal of viruses was independent of different applied filtration fluxes, but both viruses exhibited a quite different removal behavior during UF.

Without pre-ozonation, MS2 phages were reduced by UF in all replications by $\geq$ 5 LRVs and the number of phages were already below LOD. However, pre-ozonation deteriorated the removal of MS2 phages by UF. Im et al. (2018) argued that pre-ozonation hinders the destabilization of MS2 phages during coagulation, resulting in less efficient removal during UF. In this study coagulation was applied prior to UF in both experiments with and without pre-ozonation, which can physically entrap viruses within the flocs or destabilize virus particles, causing their aggregation and precipitation. However, the effectiveness of coagulation for virus removal depends on several factors, including the type and dosage of coagulant, pH, water matrix, and the characteristics of the viruses themselves (Guo and Hu, 2011; Schwaller et al., 2022)

Despite their similar particle size, PhiX174 phages behaved significantly different compared to MS2 phages during UF treatment. The operation without pre-ozonation resulted in a maximum removal of only

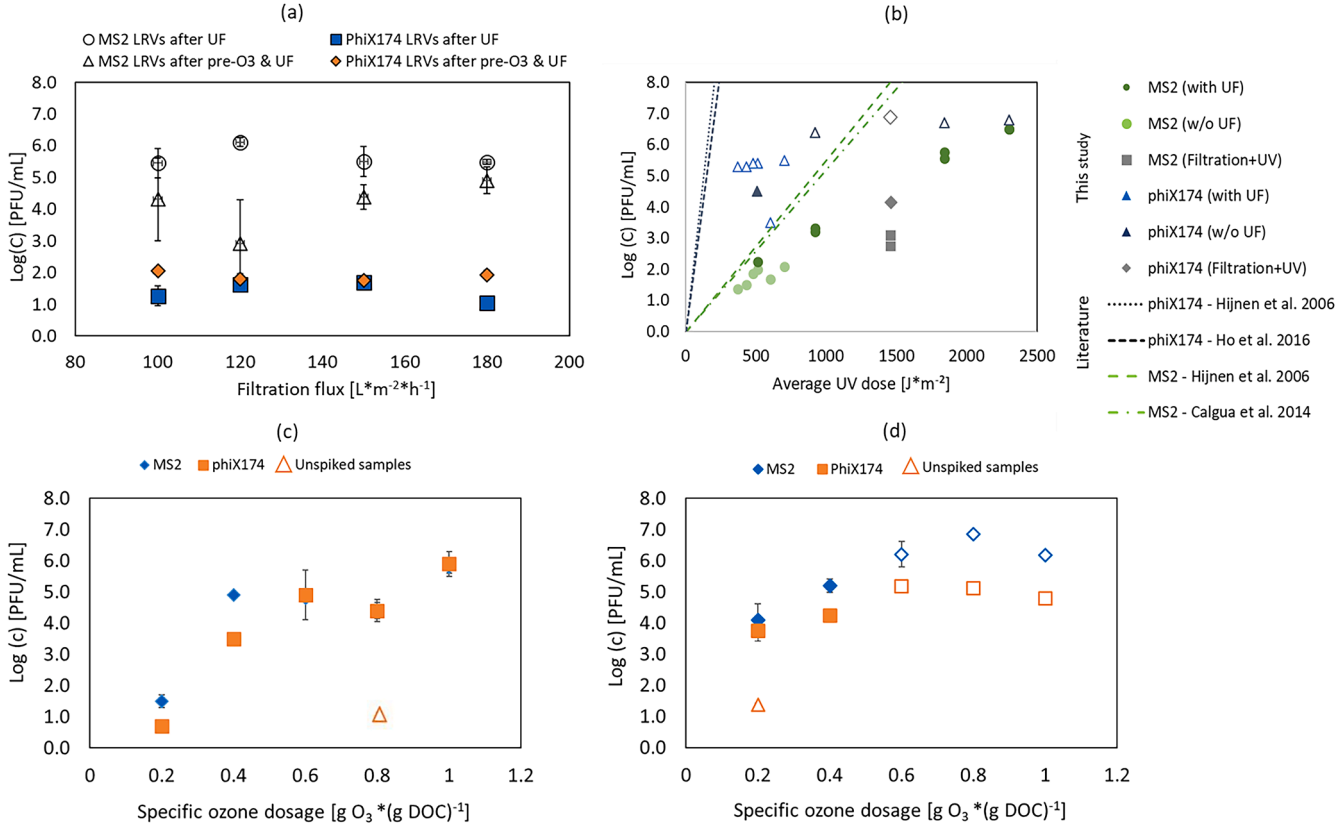

Fig. 9. (a): MS2 and PhiX174 bacteriophage removal during ceramic UF membrane filtration applying different fluxes, (b): bacteriophages behavior during UV disinfection processes under different UV fluences. The results of the microbial challenge test are illustrated with green circles for MS2 and blue triangles for PhiX174 phages. Log removal trends from other studies are displayed as dotted lines for MS2 and PhiX174 in green and blue, respectively. Spiking test results of the ozonation process without (c) and with (d) pre-treatment by UF for removing MS2 and PhiX174 bacteriophages. Empty symbols indicate removal below LOD.

1.7 LRVs. No adverse effect was observed during operating with pre-ozonation. A possible explanation is the different isoelectric point (IEP) (3.9 for MS2 phages and 6.6 for PhiX174 phages), which resulted in different surface charges as well as virus hydrophobicities (Dika et al., 2015). At neutral pH (the secondary effluent has a pH value of 7.2), MS2 and PhiX174 phages carry negative and almost neutral surface charges, respectively (Heffron et al., 2024). It should be mentioned that the measured zeta potential (ZP) of the applied ceramic UF membrane at pH 7 was around -30 to -40 mV. The electrostatic repulsion of MS2 phages from the membrane surface carrying the same surface charge (negative) could have resulted in high LRVs. The removal of PhiX174 phages during UF was quite the opposite owing to their positive surface charge and, as a result, rather dominant attraction forces between membrane surface and virus particles. Additionally, MS2 phages have a higher hydrophobicity than PhiX174 phages, which could also improve the removal of MS2 phages during UF (Dika et al., 2015). It should be mentioned that many human pathogenic viruses and even strains of the same virus have different IEPs. In case of larger viruses like human adenovirus, size also plays a role removal (Michen and Graule, 2010).

3.5.2. Determining LRVs during ozonation

The microbial challenge test for the ozonation process was performed twice. The first series of experiments were performed without the UF. After adding the UF prior to ozonation, the challenge test for the ozonation process was repeated to assess the impact of the UF as pre-treatment step on ozonation efficiency. The LRVs of MS2 phages in experiments without UF ranged between 1.5 and 5.8 for specific ozone dosages of 0.2–1.0 g O_3 (g DOC)$^{-1}$. The PhiX174 phages exhibited a similar behavior with LRVs between 0.7 and 5.9 (Fig. 9 c). Increasing specific ozone dosages from 0.4 to 0.8 g O_3 (g DOC)$^{-1}$ did not result in significantly improved LRVs. This might be due to the high concentrations of ozone scavengers during this spiking test such as TOC (> 15 mg L^{-1}), DOC (12 mg L^{-1}), TSS (> 5 mg L^{-1}), and other scavenging compounds present in secondary effluent.

During operation with ceramic UF membrane as a pre-treatment step, the ozonation disinfection efficiency was enhanced (Fig. 9 d). The ozone concentration of 0.2 g O_3 (g DOC)$^{-1}$ was applied after starting the spiking test and resulted in 4.1 LRVs – much higher than the LRVs achieved in the phase without UF. By increasing the specific ozone concentration, an almost linear increase of MS2 phages removal up to a maximum of 6.8 LRVs was observed. The concentrations of MS2 phages after applying 0.6, 0.8, and 1.0 g O_3 (g DOC)$^{-1}$ were below the LOD. The PhiX174 phages exhibited similar effects of UF pre-treatment with overall slightly lower LRVs during ozonation as compared to MS2 phages. Enhanced inactivation efficiency after UF, especially at low fluences, can be attributed to the removal of suspended solids resulting in less ozone consumption and increased ozone exposures. Moreover, the reduction of DOC (an ozone scavenger) by coagulation and UF membrane filtration by up to 30 % resulted in a decreased ozone demand. Similar findings have been discussed by von Sonntag and von Gunten (von Sonntag and von Gunten, 2012). However, potential effects of other water constituents, e.g., nitrite, cannot be ruled out. To better understand the effect of particle removal on disinfection efficiency (for seeded as well as autochthonous organisms) additional tests under controlled conditions are recommended. Comparable removal kinetics by ozone treatment were reported (Guo and Hu, 2011, Ho et al., 2016; Tseng and Li, 2006; Wolf et al., 2018).

3.5.3. Determining LRVs during BAC filtration

After spiking bacteriophages into the BAC filters, we conducted a 6-day sampling campaign to assess virus desorption from the filter material. Initially, both phages showed a sharp increase in concentration, with the peak slightly diminishing by up to one log in the effluent. When the inflow concentration returned to indigenous levels after three hours, the MS2 concentration in the effluent gradually declined, reaching the detection limit after 48 h. However, PhiX174 behaved differently, persisting in high numbers (10^3 per 100 mL) in the effluent even after six days. Routine monitoring post-spiking detected somatic phages for up to 6 weeks (10^1 per 100 mL). The detection limit was reached one month later, with subsequent samples showing no phage presence. Regarding the sum of viruses in both inflow and effluent over the observation period, the BAC filtration exhibited minimal log removal (< 1 LRV). For further details, see Figs. S3, S4 of the supplementary information.

3.5.4. Determining LRVs by UV irradiation

The removal of virus surrogates, MS2 as well as PhiX174 phages, during UV irradiation was assessed for different UV dose (ranging from 370 to 2,300 J m^{-2}) (Fig. 9 b). The MS2 phages exhibited a non-linear dose-response with decreasing sensitivity at higher LRVs, which was also reported previously (U.S. EPA, 2020). It should be noted that average UV fluences calculated using the point source summation method were used in this study, which are typically higher than RED that consider reactor hydraulics and correspond to the actual dose-response measured in standardized quasi-collimated beam tests (Pirnie et al., 2006). Despite this limitation, results show similar dose-response curves as reported in previous studies [(Leister and Hügler, 2022), 66]. Moreover, it should be considered that besides UV transmittance also feedwater turbidity can substantially affect UV disinfection efficacy. Suspended solids can protect microorganisms by shielding and through embedding into the particles, which causes tailing of dose-response curves (Beltrán and Jiménez, 2008). Previous studies reported the effect of total suspended solids on inactivation of total coliforms (Beltrán and Jiménez, 2008), but little is known on embedded C. *perfringens* and coliphages at TSS concentrations of < 10 mg L^{-1} and turbidity values of ≤5 NTU as required by the EU regulation for class A reclaimed water (EU Parliament and the Council, 2020). However, no significant effect of suspended solids was expected for the experiments in this study. This is because phages were introduced prior to individual treatment processes and turbidity values were maintained between 0.1 and 0.2 NTU (Mamane-Gravetz and Linden, 2012).

Considering the LRVs during UV irradiation, the PhiX174 phages exhibited different inactivation efficiencies compared to MS2 phages. Independent of the applied UV fluences in all cases (except for one sample), the concentration of PhiX174 phages was below the LOD. The high removal rate of PhiX174 phages has been confirmed in previous studies [(Ferrer et al., 2015), 68]. The very low sensitivity of C. *perfringens* spores in UV disinfection processes is a valid reason of applying either higher UV fluences or an additional barrier prior to UV disinfection such as UF membranes to reduce the risk of pathogens breakthrough.To ensure an effective microbial removal where UV disinfection is employed, it is essential to operate the system with online monitoring of UV transmittance, UV lamp output, and flow rate. An adjustable lamp is crucial to accommodate changes in water quality, such as decreased transmittance (U.S. EPA, 2020).

4. Conclusion

As non-potable water reuse becomes an increasingly common alternative water source for irrigation, multibarrier treatment systems play a crucial role in ensuring microbiologically safe water quality. This study evaluated two treatment trains—Train A and Train B—each with distinct strengths and challenges.

Train A, using tertiary filtration and UV disinfection (the minimum required by EU regulation 2020/741), struggled to consistently meet regulatory limits and achieve the required log removal values (LRVs) for quality class A, though it was sufficient for class B (not in direct contact with crop's edible part). To reliably meet EU regulation performance targets, UV doses should be adjusted based on reported UV sensitivities of indicator organisms. This requires validated UV reactors providing reduction equivalent doses (RED) per established protocols.

Train B, featuring ozonation, biologically activated carbon filtration, and UV disinfection, successfully met EU requirements for class A non-

potable water reuse. The applied ozone concentration of 0.6 g O_3 (g DOC)$^{-1}$ provided a barrier against microbiological contaminants, though it was less effective against spores, necessitating downstream UV disinfection.

By adding ceramic ultrafiltration membranes to Train B, microbial concentrations fell below detectable levels. While this is hygienically desirable, it complicates the validation of the treatment system's log removal capabilities. Spiking experiments were necessary, revealing cumulative reduction of >14 LRVs for MS2 and >12 LRVs for PhiX174 phages (Fig. S10), ensuring compliance with EU regulations for virus removal.

Given the distinct behaviors of bacteriophages during ultrafiltration and UV disinfection processes, it is recommended to monitor both somatic and F+ coliphages for process validation, rather than focusing on total coliphages or a single type, as suggested per current EU guidelines. Beyond defining microbial monitoring, reuse regulations should also mandate operational procedures and online measurements to ensure proper disinfection during both validation and routine operation.

CRediT authorship contribution statement

Johannes Ho: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Javad Ahmadi:** Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Carolin Schweikart:** Methodology, Investigation. **Uwe Hübner:** Writing – review & editing, Supervision. **Christoph Schwaller:** Writing – review & editing, Investigation, Formal analysis, Conceptualization. **Andreas Tiehm:** Writing – review & editing, Supervision, Funding acquisition, Data curation. **Jörg E. Drewes:** Writing – review & editing, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Jorg E. Drewes reports financial support, administrative support, equipment, drugs, or supplies, and travel were provided by Technical University of Munich. Jorg E. Drewes reports a relationship with Technical University of Munich that includes: funding grants. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

This work was funded by the German Federal Ministry of Education and Research (BMBF), for which we are especially thankful (grant # 02WV1563A). We also want to extend our thanks to Dr. Nadine Scheyer and her dedicated staff members at the WWTP Schweinfurt, as their assistance was crucial to the success of this research endeavor.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2024.122429.

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