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Influence of Particle Association and Suspended Solids on UV Inactivation of Fecal Indicator Bacteria in an Urban River

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Abstract In order to assess and accurately predict the self-purification capabilities of rivers with respect to enteric pollution, a thorough understanding of mechanisms such as dispersion, particle association, and inactivation in the water column is crucial. In this study, we firstly performed particle size distribution analyses of wastewater and investigated the *Escherichia coli* and enterococci loadings of each size fraction. It was seen that 91 % of *E. coli* and 83 % of enterococci were associated with particle sizes less than or equal to 12 μm. Particles larger than 63 μm contributed less than 1 % to overall *E. coli* and enterococci loadings. Based on these results, batch experiments were performed to investigate the effect of particle size and total suspended solids (TSS) concentration on UV inactivation of the two fecal indicator bacteria (FIB). A direct relationship between the particle size to which FIB were associated and their UV inactivation rate was noted. *E. coli* and enterococci associated with particles smaller than or equal to 12 μm were inactivated on average 2× and 1.7× faster than those associated with the larger particle fraction of 12 to 63 μm. It was additionally seen that as the TSS concentrations increased, the UV inactivation rates decreased. A tailing effect of UV inactivation was however noted at TSS concentrations above approximately 100 mg L^{-1}.

Keywords *E. coli* · Enterococci · Particle size distribution · Removal rate · Sunlight · Microbial fractionation

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1 Introduction

For urban areas relying on combined sewer networks to transport rainwater and wastewater, one major cause of surface water impairment are intense rain events. During such occasions, municipal wastewater treatment plants are unable to handle the large incoming volumetric loadings. Therefore, a portion of the sewer contents, including raw wastewater, are released directly to the aquatic environment (sanitary sewer overflows). Consequently, a sound understanding of the fate and transport of fecal pollution following such combined sewer overflows (CSOs) is essential to managing public health.

There are a variety of biotic and abiotic factors which lead to a reduction of enteric contamination in a receiving water body, including changes in temperature, pH, and salinity as well as the presence of predators and algal toxins (Auer and Niehaus 1993; Cole 1982). Moreover, physical transport processes such as sedimentation, advection, and dispersion can lead to the abatement of measured concentrations in the water column (Fries et al. 2006; Jamieson et al. 2005). Of all the factors mentioned, UV sunlight is recognized as one of the predominant mechanisms leading to inactivation of enteric microorganisms in surface waters. Especially in shallow surface waters, UV inactivation has been shown to be the principal factor influencing the survival of enteric bacteria (Schultz-Fademrecht et al. 2008; Sinton et al. 2007; Burkhardt et al. 2000; Pommepuy et al. 1992; Chigbu et al. 2005).

In fluvial systems, it is well documented that both allochthonous and autochthonous microbial communities can be highly associated with particles (Jamieson et al. 2004; Fries et al. 2008; Droppo et al. 2009; Rehmann and Soupir 2009). Particle association can provide microorganisms with benefits which they do not have when freely suspended in the bulk phase, including access to nutrients the particle may provide as well as protection from different environmental stressors such as predation (Davies and Bavor 2000; Gerba and McLeod 1976; Qualls et al. 1983; Sinton et al. 1999). Several studies have examined the degree of microbial partitioning in stormwater by using various filtration and centrifugation techniques to estimate free phase and particle-associated fractions (Jeng et al. 2005; Schillinger and Gannon 1985; Auer and Niehaus 1993; Characklis et al. 2005; Krometis et al. 2007). For example, Jeng et al. (2005) found that *Escherichia coli* and enterococci attachment to stormwater particles ranged between 22–30 and 8–12 %, respectively. This finding was similar to that of Schillinger and Gannon (1985), who identified that 15–20 % of fecal coliforms in untreated stormwater were particle associated. Investigations performed by Characklis et al. (2005) revealed that in storm samples, 30–55 % of bacterial indicator organisms were associated with particles, whereas in dry weather, this value was only 20–35 %.

The presence of particulate matter in water is known to reduce the transmission of UV light by either shading (refraction, reflection, or scattering of UV light) or encasement and results in lower UV inactivation rates of the microorganisms. There have been a multitude of studies reporting the direct correlation of particle size and/or concentration on UV disinfection efficiency of secondary wastewater effluents (Örmeci and Linden 2002; Whitby and Palmateer 1993). However, the degree to which particle association and particle size can impact UV inactivation of enteric microorganisms in flowing surface waters following CSO events is not as clear. As UV inactivation is a major contributor to overall microbial removal from the water column in shallow surface water bodies such as rivers, it is critical to understand to which particle fractions fecal indicator bacteria (FIB) are attached and how effectively UV light inactivates them.

In the case of Munich, Germany, during intense rain events, untreated raw wastewater is discharged directly into the Isar River, a body of water draining a portion of the Alps which is characterized by having no significant upstream sources of pollution. Throughout the summer months, the Isar River is a heavily frequented, recreational area and thus, there is great interest in being able to more accurately predict the fate and transport of enteric microorganisms in the river. The overall objective of this research was to investigate the influence of particle attachment and size on UV inactivation of FIB in river water following a CSO. To achieve this, firstly, the range of particle sizes found in municipal wastewater and the association of *E. coli* and enterococci with them were determined. With this information, a series of batch experiments were subsequently conducted in river water to determine UV inactivation rates of FIB associated with the different size fractions of wastewater.

2 Materials and Methods

2.1 Particle Size Distribution and Microbial Fractionation of Municipal Wastewater

To gain more insight into the occurrence of specific particle sizes in municipal wastewater from the Garching treatment plant (Germany; 27,000 PE), on four different days during dry weather, four 250-mL influent wastewater samples were collected in polypropylene (PP) bottles and stored at 4 °C until analysis was performed. A laser diffraction particle size distribution analyzer (*Partica* LA-950, Horiba Instruments, USA) was used for the measurements which all occurred within 5 h of sample collection.

In addition to particle size distribution (PSD) measurements, microbial fractionation of the wastewater was performed to better understand which size fractions contain the greatest portion of the FIB *E. coli* and enterococci. For this, 5 L of wastewater were separated into fractions by sieving through the following mesh sizes: 1,000, 500, 180, and 63 μm. From the last sieve, 25 mL of the filtrate were collected and vacuum-filtered through a 12-μm membrane filter (Schleicher and Schuell, Germany).

All sieve residues were resuspended in sterile 250 mL glass bottles with 100 mL phosphate buffered saline (PBS; pH 7). The filter was placed in a 50-mL PP sterile centrifuge tube containing 25 mL PBS after which it was treated in a sonication bath at 35 kHz for 10 min. The suspensions were finally used to determine the concentrations of *E. coli* and enterococci associated with each of the particle fractions. Moreover, the total and volatile suspended solids (TSS, VSS) concentrations of the different size fractions were determined according to German standard methods DIN 38414 (2005; S10) and 38409 (1987; H2), respectively.

2.2 Experiments to Investigate UV Inactivation Rates

Two open cylindrical batch reactors, each with a working volume of 14 L and water depth of 50 cm, were constructed from PVC and used to investigate the influence of particle association on inactivation of FIB in urban river water. An artificial sunlight spectrum with a light intensity corresponding to the annual mean radiation in Germany ($I_{290-390\ \mathrm{nm}} = 8.0\,\mathrm{W\,m^{-2}}$; Schultz-Fademrecht et al. (2008)) was generated using one overhead lamp per reactor column (Ultra-Vitalux, Osram, Germany). To approximate spring water temperatures in the Isar River, the columns were outfitted with water-chilled cooling jackets and tempered at 14 °C.

Based on the results of the microbial fractionation of wastewater (Sections 2.1 and 3.1.2), we decided to investigate UV inactivation for the following fractions: $d_p \leq 12\,\mu\mathrm{m}$, $12 < d_p \leq 63\,\mu\mathrm{m}$, and $d_p > 1{,}000\,\mu\mathrm{m}$, where d_p represents the particle diameter. The two smallest fractions $d_p \leq 12\,\mu\mathrm{m}$ and $12 < d_p \leq 63\,\mu\mathrm{m}$ were chosen as they were found to have the highest FIB loading associated with them. We also wanted to investigate larger particles sizes ($d_p > 1{,}000\,\mu\mathrm{m}$) as they are known to increase shielding and protection of attached FIB.

The same-sized sieves/filters as described in Section 2.1 were used again here to fractionate the wastewater. To obtain roughly the same FIB concentrations at the start of an experiment, different volumes of wastewater needed to be sieved/filtered. As the FIB concentration associated with $d_p > 1{,}000\,\mu\mathrm{m}$ was comparatively low, it was necessary to sieve 100 L of wastewater. For the fraction $12 < d_p \leq 63\,\mu\mathrm{m}$, 2.5 L of wastewater were first sieved and then vacuum filtered through a 12-μm filter. Finally, for the fraction $d_p \leq 12\,\mu\mathrm{m}$, we sieved 2.5 L of wastewater and collected 300 mL of the filtrate. This filtrate was subsequently vacuum filtered through a 12-μm filter. The volume of water which passed through the filter was finally centrifuged for 15 min at 6,000 rpm and 10 °C.

Depending on which particle fraction was to be investigated, either the sieve or filter residue, or the pellet was resuspended in 14 L of autoclaved water from the Isar River and added to one reactor. To facilitate removal of the particles retained on the 12-μm filter, the filters were evenly divided between eight sterilized 1-L glass bottles each filled with 1 L of autoclaved river water. The bottles were subsequently placed in a sonication bath (35 kHz) for 10 min. Following sonication, the filters were removed and the 8 L of river water/suspended particles were added to the column reactor. The remaining 6 L were finally added to achieve a working volume of 14 L. The filters were sonicated for only a short period of time so as to avoid disintegration of the particles/flocs (Tiehm et al. 2001).

Mixing of the reactor contents was achieved with a magnetic stirrer. The experiment began when the UV lamp was switched on. Two 15-mL water samples were collected in sterile PP centrifuge tubes after approximately: $t = 0, 1, 2, 5, 8, 24, 26, 32$, and 48 h. All samples were stored in the dark at 4 °C and were analyzed within 24 h. Additionally, at the end of each experiment, a 2-L water sample was collected to determine the TSS concentration.

2.3 Microbiological Analyses

Viable *E. coli* and enterococci were enumerated using the standardized microplate methods for surface water

DIN EN ISO 9308-3 and DIN EN ISO 7899-1 (ISO 1998a, b), respectively (Bio-Rad, Munich, Germany). The detection of *E. coli* is based on the presence of the enzyme beta-glucuronidase. When present in a sample exposed to the rehydrated substrate 4-methyl-umbelliferyl-beta-D-glucuronide (MUG), a fluorescent compound detectable with ultraviolet light ($\lambda = 360$ nm) is released. Microplates for enterococci detection contain the dehydrated substrate 4-methyl-umbelliferyl-beta-D-glucoside (MUD) which reacts in a similar fashion, however with the enzyme beta-glucosidase. The detection limit of the method is 3.8×10^{-1} MPN mL^{-1}.

3 Results and Discussion

3.1 Wastewater Characterization

Municipal wastewater is a critical component of stormwater overflow and thus, requires detailed characterization in order to be able to better predict the fate and transport of enteric microorganisms in rivers. As part of this study, laser diffraction analyses were performed to measure the PSD of influent municipal wastewater. Moreover, with a sieving and filtering method, the contribution of different particle fractions to overall TSS and enteric loading was determined (see Table 1) and is discussed in the following. Results are from wastewater samples collected during dry periods to avoid any influence of rainwater and surface runoff.

3.1.1 Particle Size Distribution

From the results of the laser diffraction analyses, it was seen that the smaller fractions $d_p \leq 12\,\mu$m and $12 < d_p \leq 63\,\mu$m accounted for 26 and 27 % of all particle sizes, respectively. The larger fractions $63 < d_p \leq 1{,}000\,\mu$m and $d_p > 1{,}000\,\mu$m comprised 33 and 14 % of all particle sizes found in the wastewater, respectively. Furthermore, results of the sieving/filtering technique indicated that the fraction of wastewater with a particle diameter between 12 and 63 μm contributed 45 % to the overall TSS loading. Measurements of VSS revealed that the mean organic proportion increased with increasing particle size. For example, the organic content of $d_p \leq 12\,\mu$m was only 59 %, whereas for $d_p > 1{,}000\,\mu$m, it was close to 90 %.

3.1.2 Microbial Fractionation

Sieve and filter residues were additionally analyzed for their respective FIB concentrations. The influent municipal wastewater used for the fractionation was characterized by *E. coli* and enterococci concentrations of roughly 10^4 and 10^3 MPN mL^{-1}, respectively. It was observed that the fraction $d_p \leq 12\,\mu$m contained by far the highest loading of FIB, specifically 90.6 and 83.0 % of all *E. coli* and enterococci, respectively. The other fraction that had any considerable FIB associated with it was $63 < d_p \leq 1{,}000\,\mu$m, containing 8.6 % of *E. coli* and 16.3 % of all enterococci (see Table 1). This finding is in close agreement with Jeng et al. (2005), who determined that in stormwater more than 95 % of *E. coli* and enterococci were attached to particle sizes in the range of 0.45 − 30 μm, while less than 5 % were associated with particles larger than 30 μm. Although in this study, only about 10 % of *E. coli* and 17 % of enterococci were associated with particles larger than 12 μm, this fraction should also be considered when assessing potential health risks as it provides more nutrients (Vaze and Chiew 2004) and protection from sunlight (Madge and Jensen 2006; Kollu and Örmeci 2012).

Numerical models have become indispensable tools for predicting the fate and transport of fecal contamination in surface water. As microbial water analyses are still predominately performed with culture-based techniques which typically require at least a 36 h incubation period, accurately calibrated and validated models can help accelerate decision making. The findings of Sections 3.1.1 and 3.1.2 regarding the particle size distribution and FIB association are crucial for accurately predicting the transport and sedimentation of FIB in surface waters (Servais et al. 2007; Gao et al. 2011; de Brauwere et al. 2011; Ahn 2012). Based on this fractionation data, attention needs to be focused on the fine particle fraction $d_p \leq 12\,\mu$m to better understand the fate of enteric pollution in aquatic environments following CSOs. As sedimentation would not be a primary mechanism for removal of this small fraction from the water column, there is the distinct possibility that these FIB would be able to travel greater distances downstream.

Table 1 Particle size distribution measurements of municipal wastewater and contribution of the different fractions to FIB and TSS loading

Particle fraction (μm)	Prevalence in wastewater (%)	*E. coli* adsorbed (%)	Enterococci adsorbed (%)	TSS contribution (%)	VSS (%)
$d_p \leq 12$	26	90.6 ± 7.1	83.0 ± 2.7	6	59
$12 < d_p \leq 63$	27	8.6 ± 6.5	16.3 ± 1.8	45	75
$63 < d_p \leq 1{,}000$	33	0.6 ± 0.5	0.6 ± 0.2	23	88
$d_p > 1{,}000$	14	0.2 ± 0.1	0.1 ± 0.1	26	89

3.2 UV Inactivation of FIB Associated with Different Particle Fractions

The influence of FIB attachment to different particle sizes on UV inactivation was investigated through a series of batch column experiments using natural river water and artificial sunlight ($I_{290-390\ nm} = 8.0$ W m^{-2}). Results for the first 8 h of each experiment are presented in Fig. 1.

Removal rate coefficients (k) for *E. coli* and enterococci were determined from the experimental data collected during the first 8 h of an experiment based on the assumption of first-order kinetics. In Table 2, the k values have been summarized; the influence of TSS and particle size on the inactivation rates will be discussed in the following sections.

3.2.1 Impact of Total Suspended Solids on UV Inactivation Rates

From Table 2, it can be seen that TSS concentrations differed between experiments and that for $d_p > 1{,}000$ μm, TSS levels were considerably higher than for the other two fractions. The reason for this is that FIB concentrations associated with this larger fraction were comparatively low (see Table 1) and thus, to achieve similar FIB concentrations at the start of an experiment, a greater amount of this fraction was required. As the TSS concentrations were so much higher for the experiments conducted with the fraction $d_p > 1{,}000$ μm (Table 2), UV light did not impact the inactivation rates. However, considering the fractions $d_p \leq 12$ μm and $12 < d_p \leq 63$ μm, as the TSS concentration increased, the removal rate coefficients slowed for both *E. coli* and enterococci.

In a study investigating the effect of suspended particles on UV disinfection efficiency of wastewater effluent, Qualls et al. (1985) found that as the number of particles having a diameter of 40 μm or greater increased, the number of fecal coliforms that survived also increased. Similarly, Whitby and Palmateer (1993) found a direct correlation between the suspended solids and fecal coliform concentrations. However, in their study, TSS concentrations tested ranged between approximately 10 and 65 mg L^{-1}. The fact that we observed a tailing effect at TSS concentrations of 100 mg L^{-1} and above indicates that there is a critical TSS concentration above which no further increase in inactivation occurs. This finding is of great importance when considering intense rain events, where TSS concentrations in rivers can well surpass 100 mg L^{-1}. As UV inactivation is one of the most important mechanisms leading to FIB inactivation in shallow surface waters (Schultz-Fademrecht et al. 2008; Sinton et al. 2007; Burkhardt et al. 2000; Pommepuy et al. 1992; Chigbu et al. 2005), this pronounced reduction in k at higher TSS concentrations can result in a larger geographical region being impacted by fecal contamination following a CSO.

3.2.2 Influence of Particle Size on UV Inactivation Rates

In addition to the TSS concentration, the particle size fraction with which FIB were associated also influenced k values. This effect was most evident for the fractions $d_p \leq 12$ μm and $12 < d_p \leq 63$ μm, where TSS concentrations were in a similar range. As seen

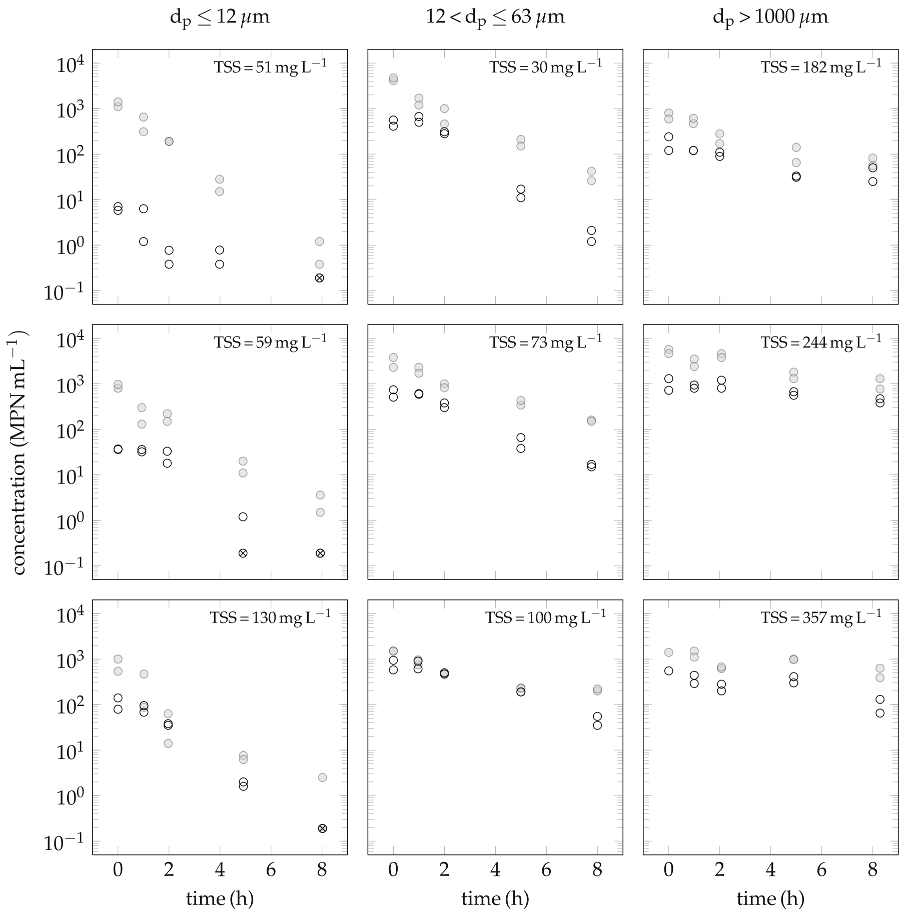

Fig. 1 Measured *E. coli* and enterococci concentrations for different particle fractions during the first 8 h of each of three groups of experiments. The corresponding TSS concentration is noted in all subplots. *Shaded circles* represent *E. coli* and *white circles* enterococci. A *multiplication symbol* indicates that concentrations were no longer detected

in Fig. 2 and Table 2, removal rates of FIB associated with $d_p \leq 12\ \mu m$ were distinctly faster than those for FIB attached to the fraction $12 < d_p \leq 63\ \mu m$. As the TSS concentrations during the experiments for $d_p > 1{,}000\ \mu m$ were significantly higher than the other two fractions, a comparison is not possible.

In a study by Madge and Jensen (2006), similar experiments were conducted with two types of wastewater effluent to investigate the effect of particle association on disinfection efficiency. The authors also found significantly slower rates of disinfection for bacteria associated with larger particles. Specifically fecal coliforms associated with particles larger than 20 µm were inactivated slower than those attached to the smaller fractions of $d_p < 5\ \mu m$ and $5 \leq d_p < 20\ \mu m$. Additionally, Qualls et al. (1985) determined that particles larger than 20 µm were more capable of protecting fecal coliforms from UV light than smaller particles. In their study, even when they included significantly more particles of $d_p < 20\ \mu m$ in the system,

Table 2 Summarized removal rate coefficients (k) for *E. coli* (ec) and enterococci (ent) with respect to particle size fractions. Coefficients of determination (R^2) are presented in parentheses next to the removal rate coefficients

Particle fraction (µm)	TSS (mg L^{-1})	k_{ec} (R^2) (h^{-1})	k_{ent} (R^2) (h^{-1})
$d_p \leq 12$	51	1.0 (0.98)	1.2 (0.64)
	59	0.8 (0.87)	0.9 (0.97)
	130	0.7 (0.98)	0.8 (0.95)
$12 < d_p \leq 63$	30	0.6 (0.98)	0.8 (0.97)
	73	0.4 (0.96)	0.5 (0.98)
	100	0.2 (0.84)	0.4 (0.95)
$d_p > 1{,}000$	182	0.2 (0.78)	0.4 (0.95)
	244	0.2 (0.89)	0.1 (0.93)
	357	0.1 (0.60)	0.2 (0.73)

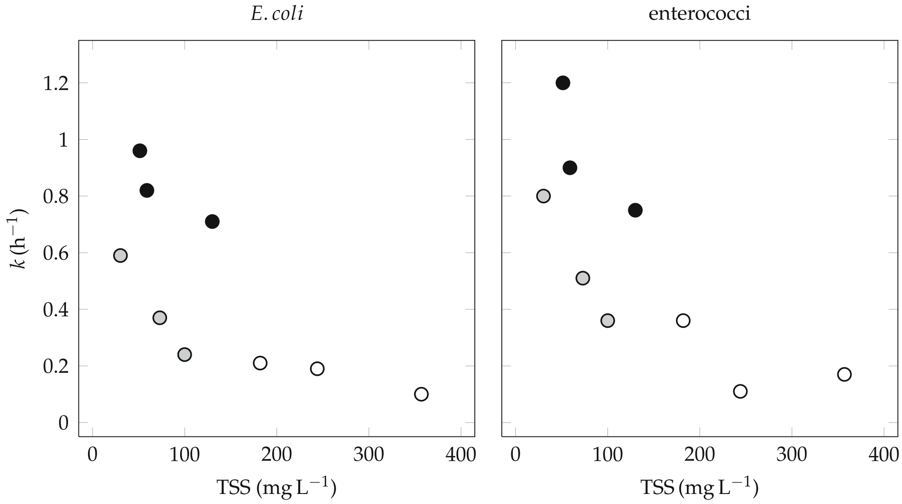

Fig. 2 Removal rate coefficients for $d_p \leq 12$ µm (*black*), $12 < d_p \leq 63$ µm (*gray*), and $d_p > 1{,}000$ µm (*white*) depicted against their respective TSS concentrations

they found that the fewer, but larger particles still provided more protection.

From the data presented in Table 1, we know that more than 90 % of *E. coli* and 80 % of enterococci present in the municipal wastewater used for this study were associated with the particle fraction $d_p \leq 12$ µm. This implies that the overall UV removal rate coefficient for FIB is dictated by this particle fraction. As *E. coli* and enterococci associated with this fraction were inactivated much faster than $12 < d_p \leq 63$ µm and $d_p > 1{,}000$ µm, UV inactivation has the potential to significantly impact overall FIB removal rates in surface water. However, CSO events are typically associated with inclement weather and therefore, at the critical moment when FIB loading is high, UV inactivation may play only a minor role in overall removal from the water column.

3.2.3 Impact of Dark and Light Conditions on FIB Inactivation

In Fig. 3, the effect of UV light (290–390 nm) on the inactivation pattern of *E. coli* and enterococci is demonstrated with the results from a 48-h experiment. Here, it is seen that during the first 8 h where artificial sunlamps generated an $I_{290-390\ \text{nm}}$ of 8 W m^{-2} at the water surface, nearly a 2-log removal of both FIB was observed. However as soon as the lamps were switched off to simulate nighttime, removal rates stagnated. As previously described in Sections 3.2.1 and 3.2.2, the degree of FIB removal during the first 8 h was dependent on the TSS concentration and particle size. Nevertheless, in all experiments, a stagnation in the removal rate was observed during the simulated night phase ($I_{290-390\ \text{nm}} = 0.0$ W m^{-2}).

CSOs are typically linked with heavy rain, little sunlight, and high TSS loads, which indeed is more closely approximated by the simulated night phases. It is questionable, however, how much we should read into this stagnation in FIB removal when $I_{290-390\ \text{nm}} = 0.0$ W m^{-2}. As these experiments

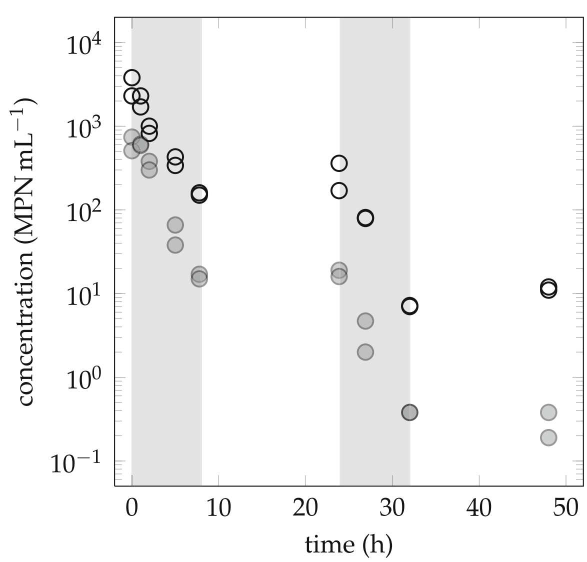

Fig. 3 UV inactivation pattern of *E. coli* (*white*) and enterococci (*gray*) attached to the particle fraction $12 < d_p \leq 63$µm throughout one complete experiment. The TSS concentration was 73 mg L^{-1}. *Shaded regions* indicate when UV lamps were on

were conducted in continuously stirred column reactors operated with autoclaved river water, crucial mechanisms such as deposition and predation did not impact FIB removal behavior in the dark. However, the advantage of such column reactors is that only by removing other factors such as sedimentation, does it become possible to directly investigate the impact of UV inactivation on FIB removal from the water column.

4 Conclusions

Raw wastewater is a critical constituent in CSOs which upon release, leads to the impairment of surface water. Following such a CSO event, the fate and transport of pathogens in rivers is to some extent influenced by the size and concentration of particulate matter. In this study, particle size distribution measurements were made of municipal wastewater. Additionally, the partitioning behavior of enteric microorganisms to the different particle fractions as well as their inactivation rates in river water were determined. The following conclusions were made:

- The particle fraction $12 < d_p \leq 63\,\mu m$ comprised more than 25 % of all particles found in wastewater and contributed to more than 50 % of the overall TSS load.
- The majority of *E. coli* (90.6 %) and enterococci (83.0 %) in wastewater were found in the fraction $d_p \leq 12\,\mu m$. This is therefore an important fraction to take into account when investigating the behavior of pathogens in urban rivers following CSOs.
- An indirect relationship was seen between the concentration of TSS and the FIB removal rate coefficient. As the TSS levels increased, the removal rate coefficients decreased. This is of significance as CSO events are associated with higher sediment loads in the water column.
- It was seen that the particle size to which FIB were associated had a direct impact on the removal rate coefficient. *E. coli* and enterococci attached to $d_p \leq 12\,\mu m$ were removed on average $2\times$ and $1.7\times$ faster from the water column, respectively, than when attached to $12 < d_p \leq 63\,\mu m$.

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