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FATE AND TRANSPORT OF FECAL INDICATOR BACTERIA IN FLUME SYSTEMS MIMICKING AN OLIGOTROPHIC RIVER

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Abstract

The impairment of surface water with enteric microorganisms as a result of combined sewer overflows is a recurring theme which plagues many cities. Such contaminations impact not only human health, but also affect the quality of life of a city's inhabitants. Many advances have been made with respect to creating accurate water quality models which can predict exceedance of fecal indicator standards. These models work to combine hydrodynamics with a varying number of mechanisms that lead to pathogen removal. However, there is still a significant knowledge gap surrounding the behavior of enteric microorganisms upon release into the aquatic environment. In the present work, lab and large-scale flumes were used to investigate various processes impacting the fate and transport of enteric pollution in oligotrophic alpine streams. Specifically, the roles of deposition, natural inactivation, and resuspension were targeted. Furthermore, the influence of suspended solids on *E. coli* and enterococci persistence in the water column as well as the potential for streambed sediments to harbor such indicator microorganisms was evaluated.

In a set of experiments conducted at the lab scale and repeated in the large-scale flume, the persistence of fecal indicator bacteria (FIB) in the water column at a constant discharge was evaluated. Interestingly, FIB removal from the water column of the lab-scale flume consistently occurred four times slower than in the large-scale flume. Although the depth:length ratios and residence times in both flumes were comparable, as were the nutrient concentrations, temperatures, and suspended solids concentrations, the significant difference in removal rates suggests that hydraulic parameters such as turbulence and bed shear stress impact persistence. In the lab-scale flume, a further investigation was performed to approximate the contributions of deposition and natural inactivation to overall FIB removal from the water column. Here it was seen that during the first 24 h, approximately 83 % of FIB removal resulted from natural inactivation (low temperature, nutrient levels), while 17 % was attributed to deposition onto the flume bed. This suggests that there should be little to no accumulation of culturable enteric microorganisms in the bed sediments of oligotrophic alpine rivers.

With the aim of evaluating the influence of suspended solids (TSS) on FIB removal from the water column, further experiments were conducted in the large-scale flume. Interestingly, at TSS concentrations of roughly $50\,\mathrm{mg\,l^{-1}}$, FIB removal from the water column occurred nearly four times slower than at a TSS concentration of approximately $20\,\mathrm{mg\,l^{-1}}$. This finding is of great importance as suspended solids levels during heavy rain events can well surpass the $100\,\mathrm{mg\,l^{-1}}$ mark, indicating the possibility of more widespread downstream pollution. Subsequent analyses of the dissolved organic carbon (DOC) associated with the suspended solids and water, indicated that TSS contributed mainly to the more readily degradable, low-weight fraction of DOC. Contrary to this, DOC associated with the water had a higher fraction of high-weight

DOC, which is considered to be less readily degradable. This result implies that the presence of TSS in the water column and continuous release of a low-weight fraction of DOC from it, could increase the persistence of suspended FIB.

Finally, experimental work was performed in both the lab and large-scale flumes to explore the potential of oligotrophic streambed sediments to harbor enteric pollution at different bed shear stresses and sediment depths. Under ideal conditions (*e.g.* no UV light or grazers/predators and low bed shear stress), *E. coli* persisted twice as long as enterococci, regardless of whether a thin (µm) biofilm was investigated or a thick (cm) sediment layer was considered. However, as soon as experiments were conducted with a realistic bed shear stress, the thicker sediment layer harbored FIB $1.5 - 2\times$ longer than the thin biofilm. Moreover, with the introduction of grazers/predators into the flume system operated at a realistic bed shear stress, *E. coli* and enterococci persistence was identical. This suggests that washout and/or grazing/predation control FIB concentrations in natural streambeds exposed to high shear.

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Preface

Selected chapters in this work have been published and/or presented in the following forms.

1. **Peer-reviewed Journal Articles**

   (a) Walters, E., Schwarzwälder, K., Rutschmann, P., Müller, E., Horn, H. 2014. Influence of Resuspension on the Fate of Fecal Indicator Bacteria in Large-Scale Flumes Mimicking an Oligotrophic River. Water Research 48, 466-477.

   (b) Walters, E., Graml, M., Behle, C., Müller, E., Horn, H. Influence of Particle Association and Suspended Solids on UV Inactivation of Fecal Indicator Bacteria in an Urban River. Water, Air, & Soil Pollution, DOI: 10.1007/s11270-013-1822-8.

2. **Oral Presentations**

   (a) Walters, E., Hille-Reichel, A., Müller, E., Horn, H. Method for Tracking Particulate COD in Biofilms, International Water Association (IWA) Biofilm Conference: Processes in Biofilms, Shanghai, China, 2011.

   (b) Walters, E., Kätzl, K., Schwarzwälder, K., Müller, E., Horn, H. Survival of Fecal Indicator Bacteria in the Sediment of an Oligotrophic River, 6^{th} International Conference on Water Resources and Environment Research: Water and Environmental Dynamics, Koblenz, Germany, 2013.

Additionally, the following manuscript has been submitted to a peer-reviewed journal.

(i) Walters, E., Kätzl, K., Schwarzwälder, K., Rutschmann, P., Müller, E., Horn, H. Persistence of Fecal Indicator Bacteria in Sediment of an Oligotrophic River: Comparing Large and Lab-Scale Flume Systems. Submitted to Water Research on June 20, 2013.

Nomenclature

Abbreviations

CFDA	carboxyfluorescein diacetate, succinimidyl ester	
cfu	colony forming units	
CLSM	confocal laser scanning microscopy	
COD	chemical oxygen demand	$(\mathrm{mg\,l^{-1}})$
CSO	combined sewer overflow	
CTAB	cetyltrimethyl ammonium bromide	
d_p	particle diameter	$(\mu\mathrm{m})$
DAPI	4',6-diamidino-2-phenylindole	
$DM_{sediment}$	dry matter content of sediment	$(g_{DM}\,\mathrm{m^{-2}})$
DO	dissolved oxygen	$(\mathrm{mg\,l^{-1}})$
FIB	fecal indicator bacteria	
$I_{290-390nm}$	ultraviolet light intensity between 290 and 390 nm	$(\mathrm{W\,m^{-2}})$
MIP	maximum intensity projection	
MPN	most probable number	
MUD	4-methyl-umbelliferyl-beta-D-glucoside	
MUG	4-methyl-umbelliferyl-beta-D-glucuronide	
OCT	optical coherence tomography	
$oDM_{sediment}$	organic content of sediment	(%)
PBS	phosphate buffered saline	
PCR	polymerase chain reaction	
PP	polypropylene	
PSD	particle size distribution	
PVC	polyvinyl chloride	
qPCR	quantitative polymerase chain reaction	
TOC	total organic carbon	$(\mathrm{mg\,l^{-1}})$
TSS	total suspended solids	$(\mathrm{mg\,l^{-1}})$
UV	ultraviolet	
VSS	volatile suspended solids	$(\mathrm{mg\,l^{-1}})$

Greek Symbols

γ	specific weight of water	$(\mathrm{N\,m^{-3}})$

λ	wavelength	(nm)
ν	kinematic viscosity	($m^2\,s^{-1}$)
τ_b	bed shear stress	($N\,m^{-2}$)
Mathematical Symbols		
$\overline{L}_F$	mean biofilm thickness	(μm)
A	cross-sectional area	(m^2)
A_{MF}	microscopic field area	(mm^2)
A_{filter}	filter area	(mm^2)
$C_{FIB,F}$	concentration of fecal indicator bacteria in biofilm	($MPN\,g_{DM}{}^{-1}$)
$C_{FIB,S}$	concentration of fecal indicator bacteria in sediment	($MPN\,g_{DM}{}^{-1}$)
C_{cells}	concentration of CFDA-labeled cells in the water column	($cells\,ml^{-1}$)
C_{ec}	*E. coli* concentration	($MPN\,ml^{-1}$)
C_{ent}	enterococci concentration	($MPN\,ml^{-1}$)
D_{FIB}	FIB dispersion coefficient	($m^2\,s^{-1}$)
I_0	irradiance at water surface	($W\,m^{-2}$)
I_z	irradiance at specific depth	($W\,m^{-2}$)
$k_{D,CFDA}$	first-order removal rate coefficient for CFDA-labeled wastewater cells (h^{-1})	
k_{att}	vertical light attenuation coefficient	(m^{-1})
$k_{D,FIB}$	first-order removal rate coefficient for fecal indicator bacteria due to deposition	(h^{-1})
k_{ec}	first-order removal rate coefficient for *E. coli*	(h^{-1} or d^{-1})
k_{ent}	first-order removal rate coefficient for enterococci	(h^{-1} or d^{-1})
$k_{I,FIB}$	first-order removal rate coefficient for fecal indicator bacteria due to natural inactivation	(h^{-1})
L_F	biofilm thickness	(μm)
N	number of thickness measurements	
n	Manning's roughness coefficient	
Q	discharge	($m^3\,s^{-1}$)
R	hydraulic radius for an open channel	(m)
R^2	coefficient of determination	
R_a	roughness coefficient	
Re_f	Reynolds number for an open channel	
S	slope	($m\,m^{-1}$)
t	time	(h or d)
T_{water}	water temperature	(°C)
u	current velocity	($m\,s^{-1}$)
V	dilution factor	(ml^{-1})
v_{dep}	deposition velocity	($mm\,s^{-1}$)
V_F	overall volume of CFDA-labeled cells detected in a sample	(μm^{-3})

V_r	retention volume	(ml)
V_{ww}	initial volume of CFDA-labeled cells added to system	(μm^{-3})
w	flume width	(m)
x	transport distance	(m)
X_{MF}	average cell number per microscopic field	
z	water depth	(m)

Chapter 1

Introduction

The problem of water contamination with human waste is one of the oldest forms of water pollution. During the Middle Ages, as towns and cities began to grow, so too did the spread of waterborne diseases. The aggregation of regional populations in urban centers, brought with it a rise in devastating epidemics due to the problems of sewage disposal and supply of clean drinking water. With the continued industrialization and urbanization that occurred throughout the nineteenth century, the transmission of waterborne diseases such as cholera and typhoid fever remained prevalent. In the midst of a cholera epidemic in London in 1854, the physician John Snow was finally able to link the spread of this disease with a contaminated drinking water supply (Snow, 1855). Once this connection was made, the advent of wastewater treatment facilities followed which over the past century, have significantly reduced the incidence of waterborne diseases in developed countries.

Although in developed countries contamination of drinking water with enteric microorganisms is a lesser issue today, surface waters continue to be plagued by fluctuating microbial water quality due to point loadings such as combined sewer overflows (CSOs) and diffuse sources, for example runoff from agricultural fields. When these compromised waters are used for recreational activities including bathing, boating, and fishing, exposure to pathogens can occur and lead to illness. For example, in August of 2010 the city of Copenhagen opted to hold a triathlon as scheduled, despite the fact that there was a torrential rain storm the day before. The result was an outbreak of gastroenteritis which infected several participants.

Many advances have been made with respect to modeling and predicting the exceedance of microbial water quality standards (Cho et al., 2010a,b; Gao et al., 2011; Bai and Lung, 2005). However, a significant knowledge gap still surrounds the survival of enteric microorganisms upon release into the aquatic environment.

1.1 Indicator microorganisms and surface water quality

1.1.1 Standard Fecal Indicator Bacteria

In water quality management and sanitary engineering practices, fecal indicator bacteria (FIB) are a frequently-used parameter for estimating the impairment of a water sample with the fecal matter of warm-blooded animals. This parameter is contingent on the fact that certain non-pathogenic bacteria are always present in the feces of these animals and if detected in a water sample, insinuate that enteric pathogens may also be present. In 1885, the German-Austrian pediatrician Theodor Escherich presented his discovery of "bacterium coli commune" better known today as *Escherichia coli* or simply *E. coli* (Escherich, 1885). Shortly thereafter in 1892, Franz Schardinger suggested the use of *E. coli* as an indicator in water monitoring (Schardinger, 1892). In 1914, the U.S. Public Health Service adopted the coliform group as an indicator of enteric pollution of drinking water and currently, it is the standard which many countries use for estimating the microbial quality of drinking and bathing waters, as well as wastewater. Ideally, such surrogates of fecal contamination should have the following attributes (Maier et al., 2009):

- Suitable for all types of water
- Present when enteric pathogens are present
- Persist longer than the most robust enteric pathogen
- Not grow in the environment
- Be detectable with a method that is simple to perform
- Their concentrations in a sample should to some extent correlate with the degree of fecal contamination
- Should be a constituent of the microflora of humans.

Examples of specific indicators of fecal contamination include total coliforms, fecal coliforms, *E. coli*, *Clostridium perfringens*, bacteriophages, and fecal streptococci. The total coliform group, which contains the species *Escherichia*, *Citrobacter*, *Enterobacter*, and *Klebsiella*, are aerobic or facultatively anaerobic, gram-negative, non-spore-forming, rod-shaped bacteria which produce gas upon lactose fermentation within 48 h at 35 °C. Although total coliforms are the oldest metric used for microbial contamination, many organisms within the group are not specific to fecal sources. Therefore, the fecal coliform group has arisen as a more close approximation of fecal contamination and includes only *Escherichia* and *Klebsiella*. These organisms are teased out of the total

coliform group based on their ability to ferment lactose with the production of acid and gas at 44.5 °C within 24 h. *E. coli* can further be distinguished by its production of the enzyme β-glucuronidase. Fecal streptococci represent a group of gram-positive bacteria which include the genera *Enterococcus* and *Streptococcus*. Enterococci account for roughly 70 - 100 % of all fecal streptococci, whereby *E. faecium* and *E. faecalis* are the two most human-specific strains (Geldreich and Kenner, 1969). Enterococci can be differentiated based on their production of β-glucosidase.

1.1.2 Limitations of using FIB as indicators of surface water quality

In many regions including North America and Europe, regulators continue to rely on FIB concentrations for their assessment of microbial water quality and subsequent decision-making. Nevertheless, there has been significant evidence that standard FIB do not always accurately reflect the presence of pathogens such as viruses, viable but not culturable bacteria, and protozoa (Jiang et al., 2001; Noble and Fuhrman, 2001; Jiang and Chu, 2004). There are more than 100 different kinds of viruses that are found in human waste which have the potential to be transmitted in the aquatic environment (Bosch, 1998). These viruses have been shown to be more resistant than many FIB to environmental conditions and treatment processes such as chlorination and UV disinfection (Harris et al., 1987; Sinton et al., 1999; Jiang et al., 2001; Lee et al., 2011).

An additional issue associated with the use of FIB is that under certain environmental conditions, they have been shown to regrow following excretion from their host (Springthorpe et al., 1993; Desmarais et al., 2002; Chandran and Hatha, 2005; Haller et al., 2009). Finally, perhaps the largest drawback of FIB is that they do not distinguish between human and animal sources. For example, high concentrations of indicator organisms force regulators to close beaches which can have detrimental impacts on a community. Such closures may however be unwarranted as the contamination may not be of human, but rather of animal origin. Weiskel et al. (1996) investigated fecal pollution in a coastal embayment and discovered that 67 % resulted from waterfowl defecation.

1.1.3 Microbial source tracking

As the major threat to human health comes from human pathogens, there has been a push in recent years towards microbial source tracking (Fong and Lipp, 2005; Field and Samadpour, 2007). Microbial source tracking methods can be categorized into two general groups, genotypic and phenotypic, and use molecular techniques either independently or in combination with culture-based analyses (Maier et al., 2009). The genotypic methods differentiate between sources of pollutants based on genetic pat-

terns of isolated bacteria from samples while phenotypic methods distinguish sources through antibiotic resistance or carbon source utilization patterns (Fong and Lipp, 2005). Both methods are further separated into those that require a library of bacterial isolates of known origin and those that do not (Maier et al., 2009). Typically, library-dependent methods are known for being quantitative, highly sensitive, and can be implemented to classify isolates from several sources (Hagedorn et al., 1999; Parveen et al., 1999). Disadvantages of these library-dependent genotypic or phenotypic methods include their time-consuming nature, the need for a large isolate database which may or may not be geographically specific, and the fact that they are prone to higher false-positive rates (Scott et al., 2002; Simpson et al., 2002; Griffith et al., 2003). With library-independent methods, there is no need to compare a sample to a database. Moreover, these methods are advantageous as they are rapid, can very accurately distinguish between human and non-human sources, and do not require a cultivation step (Scott et al., 2002; Simpson et al., 2002; Griffith et al., 2003). However, their drawbacks include the necessity of expensive equipment for the analyses as well as limited information regarding how each host-specific marker survives in the environment (Fong and Lipp, 2005; Maier et al., 2009).

Although microbial source tracking offers the hope of being able to better identify sources of fecal contamination and thus, the possibility to more accurately monitor watersheds, none of the methods have yet been recognized by the regulatory community. Significant questions still surround the temporal and geographic stability of traits and genetic sequences (Maier et al., 2009).

1.2 Fate of FIB in the aquatic environment

Enteric microorganisms are accustomed to life in the human and animal gut where nutrient levels are high and the temperature is approximately 37 °C. Upon their release into the aquatic environment, for example following a CSO, FIB are exposed to a much different set of conditions which typically do not favor their longterm survival. In FIGURE 1.1, a schematic diagram is presented which highlights the different processes impacting the fate of enteric pollution in a body of water following a CSO. In the following, a detailed discussion of the different mechanisms is provided.

1.2.1 Particle-attached vs. freely-suspended enteric microorganisms

As depicted in FIGURE 1.1, following their entry into the aquatic environment, enteric microorganisms in the water column are found either associated with particulate matter or freely-suspended. In natural systems, bacteria and most soil/sediment surfaces are negatively charged and thus, the two would typically repel each other. However,

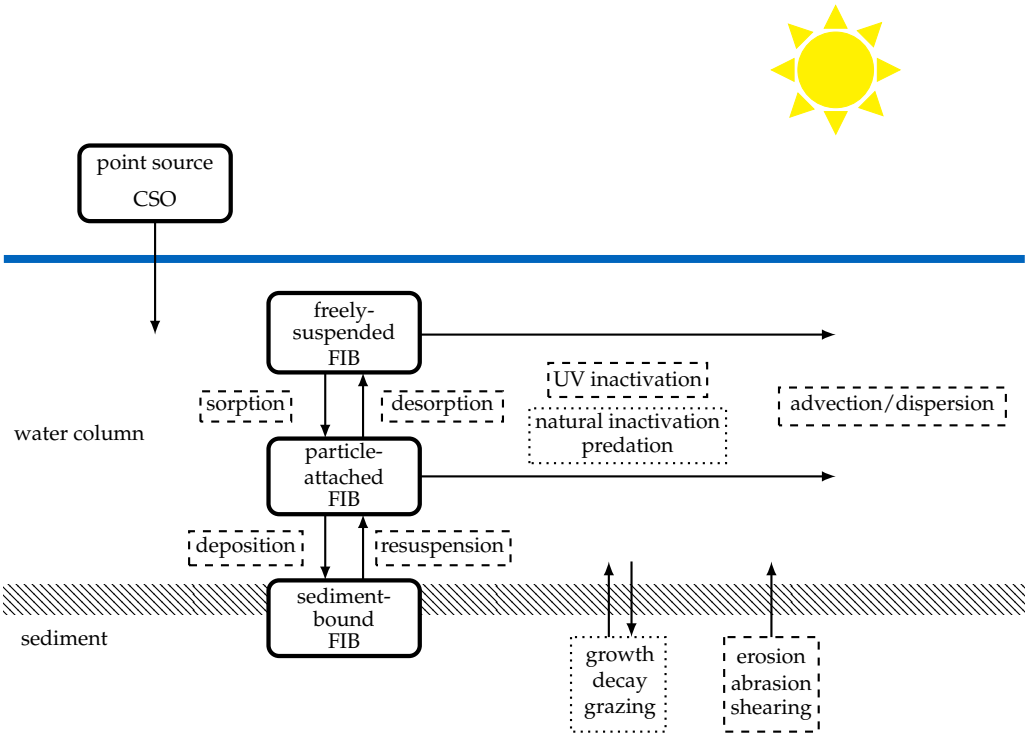


FIGURE 1.1: Schematic depiction of the fate of enteric pollution in a river following a CSO. Dashed boxes depict abiotic processes and dotted boxes biotic processes.

as outlined in Jamieson et al. (2004, 2005a), two degrees of bacterial adsorption to sediment in the aquatic environment have been identified: weak and strong attachment. Weak adsorption occurs when repulsive forces are surpassed by van der Waals forces. In such cases, bacteria are not necessarily bound to particles, but rather are only closely associated with their surfaces. The result is a type of bonding which is considered reversible. Microorganisms can also irreversibly bind to particulate matter by excreting extracellular polymeric substances (EPS) or with cellular appendages such as pili or fimbriae (Madigan et al., 2003). Such binding allows them to remain adhered to surfaces, even when exposed to the high shear stresses often found in rivers.

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 (Schillinger and Gannon, 1985; Auer and Niehaus, 1993; Characklis et al., 2005; Jeng et al., 2005; Krometis et al., 2007). For example, Jeng et al. (2005) found that *E. 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 %.

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). Particles are composed of inorganic (*e.g.* clays) and/or bioorganic (detritus, bacteria, fungus, etc.) constituents as well as water and pores (Droppo et al., 2009). In a harsh environment such as in oligotrophic rivers, 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 (Gerba and McLeod, 1976; Qualls et al., 1983; Sinton et al., 1999; Davies and Bavor, 2000). Moreover, depending on the type of particle, sorption can enhance the transport of enteric microorganisms from the water phase to the benthic zone (Droppo, 2004; Searcy et al., 2005).

1.2.2 Abiotic factors influencing microbial persistence

Microorganisms, when released into an environment in which they are not indigenous, are exposed to a variety of conditions which differ from those encountered in their natural habitat. Generally, lower nutrient concentrations and higher salinities are known to increase the rate at which FIB are inactivated (Lim and Flint, 1989; Boualam et al., 2002; Craig et al., 2004). There have been contradictory findings regarding the influence of temperature on FIB survival. It is understood that as temperatures begin to sink below optimal values, FIB metabolism also slows (Blaustein et al., 2012). However, as water temperatures begin to rise, so too does the activity of the natural microflora (McCambridge and McMeekin, 1980). Thus, experiments conducted in non-autoclaved environments have reported an indirect relationship of temperature and FIB survival (Flint, 1987; Menon et al., 2003; Craig et al., 2004). When the pressure from grazers and predators is removed, FIB survival has been shown to be higher at warmer temperatures (Flint, 1987).

Of all the factors that influence FIB survival, UV sunlight is recognized as one of the predominant mechanisms leading to inactivation of enteric microorganisms in surface waters. Especially in shallow bodies of water such as rivers, UV inactivation has been shown to be the principal factor influencing the survival of enteric bacteria (Pommepuy et al., 1992; Burkhardt III et al., 2000; Chigbu et al., 2005; Sinton et al., 2007; Schultz-Fademrecht et al., 2008). 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 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 (Whitby and Palmateer, 1993; Örmeci and Linden, 2002). However, the degree to which particle association and particle size can impact UV inactivation of enteric microorganisms in dynamic surface waters following CSO events

is not as clear. As UV inactivation is a major contributor to overall microbial removal in shallow waters, it is critical to understand to which particle fractions fecal indicator bacteria (FIB) are attached and how effectively UV light inactivates them.

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. Likewise, Whitby and Palmateer (1993) found a direct correlation between the suspended solids and fecal coliform concentrations. However, in their study total suspended solids (TSS) concentrations tested ranged between approximately 10 and 65 $mg\,l^{-1}$. As suspended solids concentrations in rivers during and immediately after heavy rain events can be above 100 $mg\,l^{-1}$, a direct correlation between UV light and inactivation may no longer exist in such turbid waters.

Madge and Jensen (2006) conducted similar experiments 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, they found that the fewer, but larger particles still provided more protection.

1.2.3 Biotic factors impacting microbial persistence

Significantly more research has been done with respect to the effect of abiotic parameters on survival of allochthonous microorganisms in non-indigenous environments. However, there are a few key biotic processes including predation, competition with autochthonous microbiota (Enzinger and Cooper, 1976; McCambridge and McMeekin, 1980), and lysis from lytic bacteria as well as bacteriophages which affect microbial persistence in the aquatic environment (Flint, 1987; González et al., 1992).

Predation/grazing by nanoflagellates, ciliates and macroinvertebrates is perhaps the most predominant biotic factor controlling bacterial densities in aquatic systems (Porter et al., 1985; Barcina et al., 1991; González et al., 1992; Menon et al., 1996; Barcina et al., 1997; Menon et al., 2003). In streams, the benthic biofilms/periphyton account for a significant fraction of the total organic matter present and are a known food source for stream invertebrates (Bärlocher and Murdoch, 1989; Decho and Lopez, 1993; Findlay et al., 1993; Boulton et al., 1998). As a result, grazers are prevalent in this region and have been shown to reduce biomass and impact the composition of algal species located therein (Underwood and Thomas, 1990; Feminella and Resh, 1991; Lawrence

et al., 2002). Both consumptive (*e.g.* ingestion) and non-consumptive (*e.g.* from foraging) losses due to snails, ostracods, and other herbivores have been cited in periphyton biomass and algae reduction in streams (Lamberti et al., 1987; Scrimgeour et al., 1991; Lawrence et al., 2002). For example, Scrimgeour et al. (1991) noted that the presence of larval mayflies led to significant non-consumptive losses of benthic biofilm.

In a study by Hall et al. (1996), the authors investigated the uptake length of fluorescently-labeled bacteria in a stream reach as well as the impact that filter-feeding organisms can have on retention of these particles. Here *Simulium*, a filter-feeding blackfly larva, was found to be accountable for more than 90 % of the overall invertebrate ingestion. Other filter feeders investigated in the study relied on mesh nets (42 - 302 µm in size) to retain particles in the water column. *Simulium* have brush-like structures on their heads called cephalic fans, allowing them to capture submicron-sized particulate matter in the water (Wotton, 1976). In their study, Hall et al. (1996) found that *Simulium* had little impact on the bacterial uptake length when compared to physical processes. However, as blackfly abundance was low in their investigated stream reaches, they suggested that in streams with higher densities the potential for blackflies to regulate bacterial transport would increase.

DeBruyn and Rasmussen (2002) performed studies in a large, fast-flowing temperate river in an attempt to quantify the degree of assimilation of sewage-derived particulate organic matter by riverine benthos. The authors used stable carbon and nitrogen isotopes to examine the influence of an upstream municipal wastewater treatment plant on the benthic food web of the river. Although they did not find an accumulation of the particulate matter in the bed sediments, they did find that more than 60 % of the carbon and nitrogen in the benthic organisms could be traced back to the sewage. Additionally Rauter et al. (2005), who evaluated the fate and transport of sewage particles in a stream, found that particulate organic matter of sewage origin supports streambed metabolism. These findings reaffirm that the benthic zone can influence microbial persistence in the aquatic environment.

1.2.4 Transport and deposition

An abundance of literature exists, which has shown that the majority of enteric microorganisms in the aquatic environment are associated with particulate matter. As a result of this association, suspended solids can act as vectors, enhancing the transport of contaminants both longitudinally and vertically within streams. Therefore, the ability to accurately predict the transport of fine and especially very fine particulate matter is key to estimating microbial water quality after contamination.

The particle transport distance is a parameter used to define the mean longitudinal distance a particle travels after introduction or re-introduction into the water column until it is deposited. Newbold et al. (1981) asserted that by assuming turbulence keeps

particulate matter well-mixed in the water column, the concentration of particulate matter decreases exponentially with the downstream distance traveled. By linearizing this function, the average downstream distance traveled is understood to be the inverse of the slope. In their study on particle settling in flowing water, Reynolds et al. (1990) confirmed transport distance to be inversely related to water depth in turbulent systems where the entrainment criterion is exceeded.

The deposition velocity is an additional parameter used, which describes the vertical movement of particulate matter. It is obtained by dividing the width specific discharge (product of water depth and mean velocity) by the particle transport distance. As pointed out by Thomas et al. (2001), this represents a more useful parameter for evaluating the influence of different channel and/or benthic traits on the flux of suspended solids from the water column to the streambed.

Often, the vertical removal of particulate matter from the water column is calculated with a form of Stokes' law, which focuses on the role of gravitational settling in quiescent waters. In surface waters there is a large variety of particulate matter found in suspension. As these particles all have different densities, sizes, shapes, roundness, and surface textures, it is difficult to generalize with one settling rate.

Several studies have been performed to compare such particle fall velocities with calculated deposition velocities (Cushing et al., 1993; Hall et al., 1996; Thomas et al., 2001; Rauter et al., 2005). In a study by Thomas et al. (2001), the authors conducted field experiments with radiolabeled natural particles to investigate the influence of particle size on seston deposition in streams. For the three different particle size fractions considered, the deposition velocity was seen to increase with particle size. Moreover, they noted that the ratio of deposition to fall velocities decreased with increasing particle size. For the very fine particulate organic matter fraction (15 - 53 µm), the role of gravity on settling was minimal, which suggests that other processes control deposition of such small particles. They proposed that particle size begins to influence seston deposition at sizes between approximately 50 an 100 µm.

In a paper by Jamieson et al. (2005a), findings related to the transport and deposition of *E. coli* associated with particles in two natural streams have been presented. The authors considered two particle size fractions, 45 - 75 µm and 75 - 125 µm. This was the first attempt to experimentally determine deposition rates for sediment-associated bacteria in flowing water. Here it was seen that the calibrated settling velocities were two orders of magnitude lower than the predicted fall velocities. This they attributed to high streambed shear stresses which limited the number of particles that could actually bind with the bed without being re-entrained.

1.2.5 Potential for the benthic zone to harbor pathogens

Knowing that deposition of FIB out of the water column and into the benthic zone contributes to overall FIB removal from the water column, it is important to understand the potential for bed sediments to promote persistence. With this in mind, Burton et al. (1987) performed a series of experiments in flow-through, lab microcosms to study the survival of several resistance-labeled bacterial pathogens in different freshwater sediments. Over a two-week period, the authors noted extended survival of FIB in the sediments, with survival rates for *E. coli* ranging between 0.7 and 0.9 h^{-1}. They additionally found that persistence was greater in sediments containing at least 25 % clay, however all attempts to correlate survival with total organic matter were unsuccessful. The authors attributed this to the varying nature of organic matter and the wide variety of other environmental factors which impact survival.

As part of a study by Davies et al. (1995), the authors performed long-term, *in situ* microcosm experiments to better understand the survival of FIB in both freshwater and marine sediments. After nearly 60 d, a 2-log reduction in fecal coliform and fecal streptococci had been seen, however concentrations were still measurable. The authors additionally conducted lab-scale microcosm experiments to better understand the influence of predators on FIB persistence. In the absence of predators, they observed a net growth of FIB. However, as soon as protozoa were introduced into the system a net overall decay occurred. This suggested that concentration reductions seen in the *in situ* experiments resulted due to the imbalance between growth and predation.

More recently, Droppo et al. (2009) conducted field measurements to examine the association of FIB with TSS and surficial bed sediments in a river mainly transporting fine-grained cohesive sediments. On average, the authors found *E. coli* concentrations in the bed sediments to be 5 - 6 orders of magnitude higher than what was detected in the river water. Interestingly, they also compared FIB concentrations in the bed to those found in the suspended solids. It was initially hypothesized that due to permanent deposition of such flocs onto the streambed, FIB concentrations in the bed should have been higher than those associated with the suspended solids. However, *E. coli* concentrations connected with the TSS were in fact higher. The authors suggested a lower survival rate in the bed sediments due to unfavorable physico-chemical conditions. To support this, they reported finding a higher proportion of dead cells in the bed sediments compared to the TSS.

As it has been repeatedly shown that bed sediments can harbor FIB, current monitoring practices which only measure concentrations in the water column, are potentially underestimating the overall threat. McDonald et al. (1982) presented an interesting case where an artificial release of water from a reservoir was performed so as to have adequate volumes of water for the day's slalom canoeing event. During the rising limb of the hydrograph, *E. coli* and total coliform concentrations were 10 - 30 times

higher than baseflow values, which they associated with re-entrainment of FIB from the streambed. Thus, as this compartment can have significant implications on water quality, it deserves attention in monitoring and modeling efforts.

1.2.6 Resuspension

As streambed sediments have been shown to harbor enteric microorganisms much longer than the overlying water column, there is the threat that their remobilization or resuspension can have unexpected, detrimental impacts on water quality. Within the past 35 years there have been several attempts by researchers to more closely examine the resuspension of bacteria-laden streambed sediments. McDonald et al. (1982) performed a series of experiments where streamflow was increased by releasing water from a reservoir so as to mimic a high-flow event. In doing so, the authors were able to prevent the input of FIB to the river due to surface runoff and thus, only examined FIB from the streambed. It was seen that in the absence of precipitation, total coliform and *E. coli* concentrations increased more than $10\times$. McDonald et al. (1982) concluded that there are two stores in a catchment which can contribute to enteric contamination, the land and in-stream stores, and that the in-stream supply is finite.

In a field study, Nagels et al. (2002) aimed to compare the contributions of surface runoff and remobilization of in-stream FIB stores on the microbial water quality of an agricultural stream. They observed roughly a $2\times$ increase in the *E. coli* concentration in the water column and concluded that resuspension of bed sediments due to flood currents may be the predominant source of FIB during natural flood events. Muirhead et al. (2004) conducted experiments where floods were artificially simulated during dry weather to avoid input of land-associated FIB. Here again, a two-fold increase of *E. coli* in the water column was observed. By performing triplicate flooding events in series, the authors estimated the in-channel supply of *E. coli* to be roughly 10^8 cfu m^{-2}.

In a 2005 paper published by Jamieson et al., the authors attempted to go one step further and quantitatively link both the timing and degree of resuspended bacteria with the hydraulic conditions and bed sediment characteristics. It was observed that in streams characterized by a mixture of cohesive and non-cohesive sediments, transport of the cohesive fraction was predominantly responsible for movement of sediment-associated *E. coli*. A critical shear stress of $1.7\,N\,m^{-2}$, comparable to that for erosion of cohesive sediments, was deemed the point at which resuspension of in-channel FIB stores occurred. Most recently, Cho et al. (2010b) published the findings of their experimental and modeling efforts regarding the release of *E. coli* from a streambed. Following artificial high-flow events, the authors observed *E. coli* spikes in the water column followed by long tails in the concentration. This suggests low settling rates and thus, the possibility of long downstream transport distances. Moreover, the authors mentioned the presence of "hot spots" or high spatial variability in concentra-

tions of streambed-associated bacteria. Such a heterogeneous distribution of FIB in bed sediments proves difficult to capture with sampling efforts and is only manifested in pollutographs, which are unable to pinpoint a source's location. Nevertheless, as resuspension of FIB-laden bed sediments can influence the overlying water quality, it is essential to consider this compartment as a source in surface water quality models (Thomann and Mueller, 1987).

1.3 Objectives of this work

For decades attention has been placed on understanding the fate and transport of enteric pollution in surface water. Although significant strides have been made in better predicting microbial water quality, there are still knowledge gaps which surround the topic.

Extensive work has been done to examine the persistence of enteric organisms and viruses in a variety of fresh and saltwater systems. Nevertheless, as their survival is dependent on numerous biotic and abiotic factors unique to each body of water, it is not always possible to apply findings from one waterbody to make predictions in another. In CHAPTER 3, results from a series of experiments conducted in lab-scale flumes mimicking an oligotrophic river are presented which aim to shed light on FIB removal from the water column of the Isar River. Through use of a combination of culture-based and microscopy techniques, an attempt has been made to identify the individual contributions of inactivation and deposition on overall removal from the water column.

The role of streambed sediments in promoting FIB persistence has been shown for a variety of different systems and is linked in part with the higher nutrient levels present in sediment as well as the limited penetration of UV light to the benthic zone. In streams, the flow of water creates shear stresses at the benthic bed which are greater than those in the water column. Few studies however have attempted to examine the role of bed shear stress on FIB persistence in bed sediments. Therefore, CHAPTER 4 evaluates the fate of FIB in a lab and large-scale flume characterized by a low and typical (for the Isar River) bed shear stress, respectively. Finally, through use of ceramic tiles and substratum cages the ability of a thin biofilm (µm) versus a thick (cm) layer of sediment to harbor FIB is compared.

In recent years, attention has been placed on linking hydrodynamic conditions of a waterbody with the persistence of FIB. With the knowledge that benthic sediments can act as a reservoir of pathogens, different research groups have attempted to investigate the re-entrainment of FIB back into the water column due to sudden changes in discharge. In CHAPTER 5, results from two series of experiments conducted in a large-scale flume are presented. Firstly, standard removal rates of FIB from the water column

of an oligotrophic stream were determined and are compared to those determined in a lab-scale flume. Subsequent experiments were then conducted to elucidate the relationship between an increase in bed shear stress on suspended solids concentrations and FIB persistence in the water column.

In both wastewater treatment as well as the aquatic environment, association of FIB with particulate matter has been shown to enhance their persistence. Not only can particles act as an added nutrient source and mode of transport for normally buoyant microorganisms, they can also shield FIB from harmful UV rays. As UV inactivation plays a major role in removal of FIB from shallow surface waterbodies, it is important to understand to which particle fractions FIB are attached and how rapidly UV light inactivates them. In CHAPTER 6, a characterization of the wastewater used in this work is provided, which specifically informs about the attachment of FIB to different particle sizes. Moreover, the chapter presents the results of batch experiments targeted at investigating the influence of particle size on UV inactivation of FIB.

In summary, the intention of this Ph.D. thesis is to examine in detail the fate and transport of FIB in different compartments of an oligotrophic, alpine river following a CSO. Specifically, it evaluates FIB persistence in the water column of both a large and lab-scale flume mimicking the Isar River. Moreover, it examines the capability of streambed sediments to harbor *E. coli* and enterococci and thus, their potential to act as a reservoir of pathogens. Finally, the influence of suspended solids on FIB survival in the water column is studied as well as the role particulate matter plays in hindering UV inactivation of FIB.

Chapter 2

Materials and Methods

2.1 Wastewater and river water used

For all experimental work performed, the following municipal wastewater and river water was used.

Wastewater

All wastewater used in this study was taken from the municipal treatment plant situated in Garching, Germany, which has a population equivalent of 27,000. Before use, the wastewater was mechanically screened (1.5 mm) whereafter it went through a primary clarification step . During periods of dry weather, the wastewater has roughly 300 mg l^{-1} COD, 100 mg l^{-1} TOC, 70 mg l^{-1} TKN, and a TSS concentration of 50 mg l^{-1}. *E. coli* and enterococci concentrations in the wastewater are typically 10^4 and 10^3 MPN ml^{-1}, respectively.

River water in lab-scale flume

All lab-scale experiments were conducted with water collected directly from the Isar River at a distance of 128.4 km downstream, just north of Munich, Germany. The Isar River is a 295 km long stream which drains a portion of the Alps, runs through Munich, and finally empties out into the Danube River. The river has a gravelly bed with a D50 of approximately 8 mm. The river is predominately surrounded by meadows and grassy regions and thus, diffuse contamination through farming and agriculture does not significantly influence the river's water quality. Nevertheless, the quality of the water decreases downstream due primarily to the fact that several wastewater treatment plants discharge directly into the river. Nevertheless, the Isar River scores a II in the German Water quality index, indicating moderate contamination.

Throughout the summer months, the river is a heavily-frequented recreational area. To increase the hygienic quality in the Munich region during this time, wastewater

treatment plants between Bad Tölz and Moosburg introduced an additional UV disinfection step in 2005. As a result, from mid-April until the end of September the river typically exhibits bathing water quality as characterized by the EU Bathing Water Directive (The European Parliament and Council of the European Union, 2006). However, during periods of intense rain events when wastewater treatment plants are forced to perform combined sewer overflows, the water quality can drastically fluctuate.

The water temperature during the summer months is approximately 17 °C, COD is about 10 mg l^{-1}, and TSS levels are between 10 and 15 mg l^{-1}. As suggested by Singer et al. (2006), prior to using the river water in lab-scale Flume A it was sieved (100 µm) to eliminate larger particles and insect larvae.

River water in large-scale flume

Large-scale experiments performed at the TUM Test Station in Obernach, Germany were conducted with water from the Obernach River, a branch of the Isar River that drains into Walchensee. This diversion occurs at approximately river kilometer 250, or 45 km downstream, and was done so to generate hydroelectric power. The average water temperature during the summer months is roughly 11 °C and oxygen saturation is nearly 100 %. The river water typically has a COD concentration of < 5 mg l^{-1} and a pH of 8; TSS concentrations are below 10 mg l^{-1}. This water was not filtered prior to use.

2.2 Experimental systems

To explore the fate and transport of FIB in the Isar River, two flume systems and one column reactor were utilized and are described in more detail in the following sections. Firstly however, the hydrodynamic parameters used to characterize the flumes are introduced.

2.2.1 Hydrodynamic parameters for open channels

Reynolds number

To describe the hydrodynamic conditions in the two flume systems, the Reynolds number for an open channel, Re_f, was used and calculated according to Singer et al. (2006) as follows:

$$Re_f = u\frac{R}{\nu}, \tag{2.1}$$

where u is the current velocity, R is the hydraulic radius, and ν is the kinematic viscosity. The hydraulic radius for an open channel can be determined with the flume width, w, and water depth, z, according to the following equation:

$$R = \frac{w \cdot z}{(w + 2z)}\,. \tag{2.2}$$

Dingman (1984) proposed that for open channels, the transition from laminar to transitional flow occurs at an Re_f of 500 and the onset of fully turbulent flow is associated with an $Re_f > 2000$. By injecting rhodamine into their lab-scale flume systems, Singer et al. (2006) found laminar, transitional, and turbulent flow occurred at an Re_f of 320, 798, and > 1917, respectively.

Bed shear stress

To estimate bed shear stress in the flume systems, a similar method as presented in Jamieson et al. (2005b) was used. Firstly, Manning's equation was implemented to express the discharge with respect to the flume geometry:

$$Q = \frac{1}{n} R^{2/3} S^{1/2} A\,, \tag{2.3}$$

where Q is the discharge ($m^3\,s^{-1}$), n is the Manning's roughness coefficient, R again is the hydraulic radius (m), S is the slope in the flume ($m\,m^{-1}$), and A is the cross-sectional area of flow (m^2). A roughness coefficient of 0.031 was used and the slope of the flume bed was approximately $0.002\,m\,m^{-1}$. The bed shear stress can be calculated with the following equation:

$$\tau_b = \gamma R S\,, \tag{2.4}$$

where τ_b is the bed shear stress ($N\,m^{-2}$) and γ is the specific weight of water ($N\,m^{-3}$).

By combining Equations 2.3 and 2.4, the bed shear stress can be expressed as a function of discharge:

$$\tau_b = \gamma S^{1/4} \left(\frac{n}{A}\right)^{3/2} Q^{3/2} \tag{2.5}$$

2.2.2 Indoor lab-scale Flume A

Lab-scale Flume A (FIGURE 2.1) was fabricated with PVC and had a working length of 1.2 m, width of 10 cm, and water depth of 4 cm. A stainless steel plate (mesh size 0.5 cm) was positioned at the inlet in order to smooth the inflowing water. An overflow weir was used at the end of the flume to maintain a water depth of 4 cm. Water exited the flume and flowed into a 15 l recycle tank that was tempered at 12 °C. From the recycle

tank, the majority of the water was returned back to the flume, however a small fraction could be spilled through the overflow outlet. Thus, when operated in fed-batch mode, the volume of water in the flume remained constant.

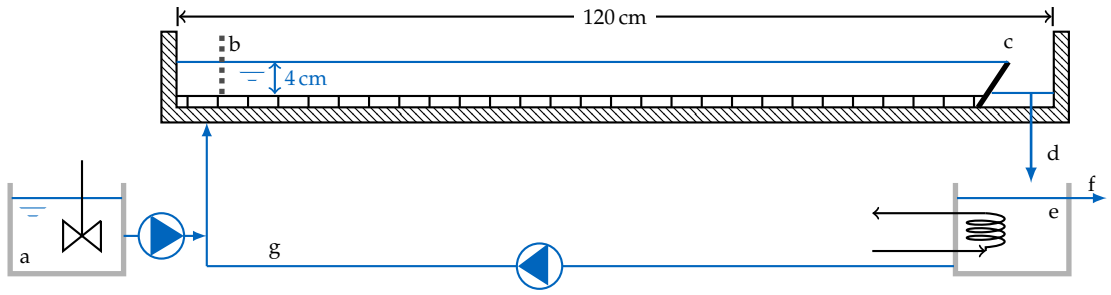


FIGURE 2.1: Schematic drawing of one lab-scale flume as seen from the side and comprised of: a. 20 l storage tank, b. perforated stainless steel plate, c. overflow weir, d. outlet, e. tempered 15 l recycle tank, f. overflow outlet g. recycle stream

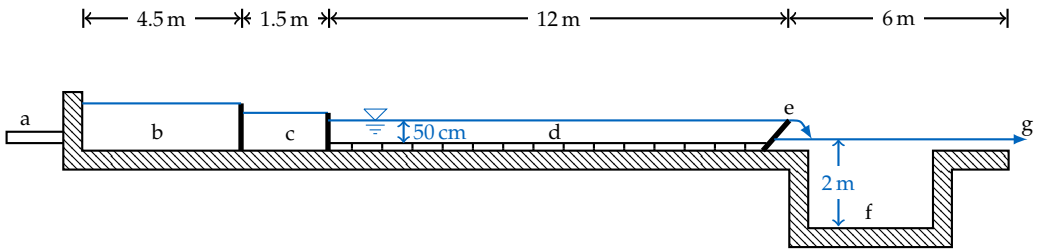
Flow was generated using a magnetic gear pump (MDG-M15S3B, PAT Niemzik, Hann, Germany). A peristaltic pump (Ecoline VC-MS CA8-2, ISMATEC SA, Glattbrugg, Switzerland) was used to continuously dose fresh river water to the system; the volume of the system (15 l) was exchanged four times per day during fed-batch operation. Three sun lamps (Ultra-Vitalux, Osram, Germany) were mounted above the flume to achieve a UV intensity, $I_{290-390\,nm}$, of approximately $8\,W\,m^{-2}$ at the water surface. To create day-night cycles, the sun lamps were switched on for 8 h per day.

Experiments in Flume A were conducted at an Re_f of roughly 1100, indicating transitional flow. The bed shear stress was approximately $0.3\,N\,m^{-2}$, which is considerably lower than typical values for the Isar River ($\approx 10\,N\,m^{-2}$).

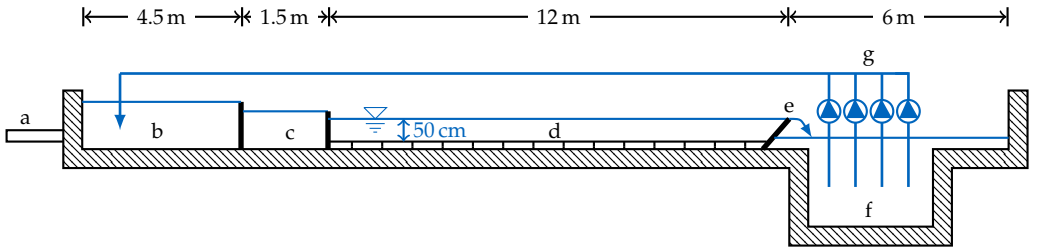
2.2.3 Outdoor large-scale Flume B

FIGURE 2.2 depicts the large-scale flume system used in this study, which could be operated in either (a) flow-through or (b) recycle mode. The flume is located at the Hydraulic and Water Resources Engineering Test Station in Obernach, Germany (Oskar von Miller Institute, Technische Universität München). Flume B was constructed out of concrete (length 12 m, width 0.5 m, water depth 0.5 m) and had a working volume of approximately $13\,m^3$.

As already mentioned, the water used in the flume was taken from the Obernach River, a branch of the Isar River. After a weir in the Obernach River, a portion of the river water was directed towards the grounds of the Test Station. The water was stored in a reservoir where a significant amount of sand and silt settled out of the water. By exploiting hydrostatic pressure, the water was directed from the reservoir to the inlet basins of the four flumes. In order to reduce turbulence, the water was subsequently fed into settling basins via overflow weirs; the settling basins tapered



(a) flow-through mode



(b) recycle mode

FIGURE 2.2: Schematic drawing of large-scale flume system in Obernach. Upper flume is in flow-through mode and bottom flume is in recycle mode. a. inlets, b. inlet basins, c. settling basins, d. flumes, e. overflow weirs f. collection basins, g. outlet or steel recycle pipe with four pumps

down to the flume inlet width of 0.5 m. Upon exiting the 12 m long flume, the water flowed into a collection basin which could either be left open for flow-through conditions (FIGURE 2.2a) or closed when recirculation was desired (FIGURE 2.2b). To achieve recirculation, four submersible pumps were attached to a steel recycle pipe and submerged into the closed-off collection basin. The water was thereby pumped back to the settling basin. The volume of water entering the flume was regulated with a slide gate at the flume inlet. The water depth was controlled by overflow weirs located directly before the collection basins.

The flumes were carefully designed to mimic conditions within the upper reaches of the Isar River. During the summer months in the Munich region, the river has a flow velocity between 0.5 and 2 m s^{-1}. As UV inactivation is perhaps the greatest contributor to overall FIB removal and light attenuation occurs over the water depth, it was desirable to have a flume that was approximately 50 cm deep. By using both natural substratum from the Isar River and fresh water from the Obernach River, scaling effects were minimized. The gravel which was used for the substratum in the flumes was removed from the Isar River, approximately 3 km upstream of the Test Station. The granulometric composition was not altered so that the D50 was between 7.5 and 9.5 mm and the D90 between 35 and 50 mm. Over time fine sediments also accumulated on the flume bed. The characteristics of these fine sediments are presented in TABLE 2.1.

The granulometric composition of the bed sediment was not scaled-down as the

TABLE 2.1: Analysis of the fine sediment found in the bed of large-scale Flume B. The classification refers to coarse (C), medium (M), or fine (F) sand (Sa) or silt (Si), or clay (Cl).

particle size (μm)	classification	percent fraction (%)
630-2000	CSa	0.4
200-630	MSa	0.8
63-200	FSa	0.8
20-63	CSi	20.4
6.3-20	MSi	46.8
2-6.3	FSi	22.3
<2	Cl	8.7

intention was to operate the flumes with a bed shear stress typical for the Isar River during dry weather flow (8 - 10 $\mathrm{N\,m^{-2}}$). The bed shear stress is an important parameter when considering deposition and resuspension in rivers. As the water depth in the flumes was held constant, the bed shear stress was altered by varying the water velocity. The water velocity was measured periodically during the experiments with an acoustic doppler velocimeter (Vectrino II, Nortek, Rud, Norway). The discharge in the flume could be adjusted to achieve bed shear stress values between 2 and 11 $\mathrm{N\,m^{-2}}$. Although bed shear stresses typical of high flow events in the Isar River could not be achieved in the flume, values were well above the critical stresses required to erode cohesive sediments. With an Re_f of approximately 1.0×10^5, the flow in large-scale Flume B was clearly turbulent.

2.2.4 Benthic substrata

In order to more closely examine the interaction of FIB with the benthic biofilm of an oligotrophic river, unglazed ceramic tiles and substratum cages were used in both Flumes A and B. In FIGURE 2.3, the two types of substrata can be seen.

Ceramic tiles

Unglazed square ceramic tiles having dimensions of 2.5 cm $\times$ 2.5 cm $\times$ 0.6 cm were used to perform investigations with a flat, thin benthic biofilm (200 µm). Experiments with the tiles were performed in both Flume A and Flume B. For experiments in Flume A, the entire flume bed was paved with the tiles. Given the large dimensions of Flume B, only 30 tiles were used. They were glued to stainless steel holders which could be easily removed from the flume bed during sampling.

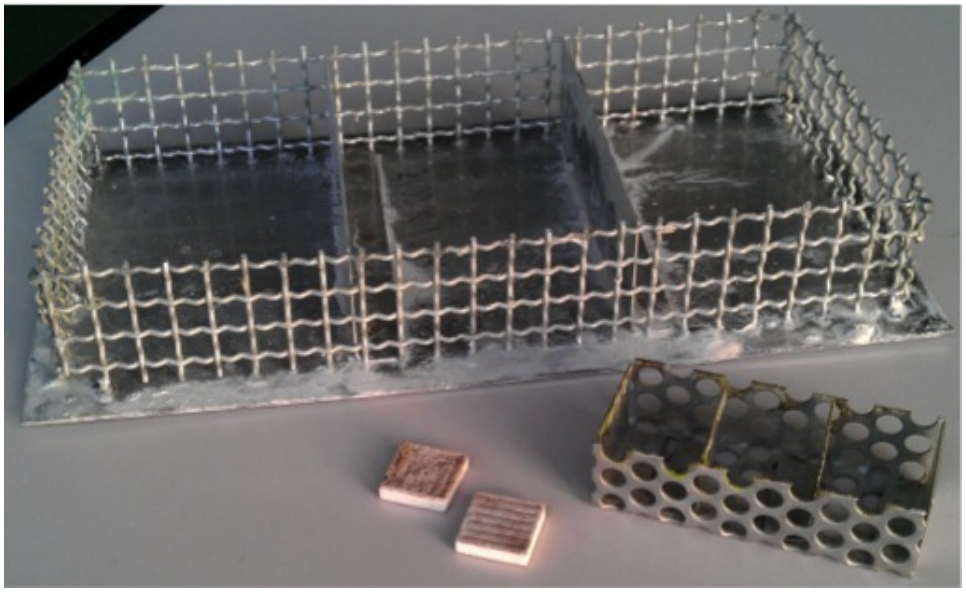


FIGURE 2.3: Substratum cages and tiles used in the study. Small cages (volume 135 cm^3) were used in Flume A and large cages (volume 1350 cm^3) in Flume B. Ceramic square tiles with the dimensions 2.5 cm $\times$ 2.5 cm were used in both flumes.

Substratum cages

Lab and large-scale substratum cages were also used to better understand the fate of FIB in a thicker layer of sediment (cm). Lab-sale Flume A was outfitted with thirteen identical cages having a length of 4.5 cm, width of 10 cm and depth of 3 cm. The cages were constructed from stainless steel having a mesh size of 0.8 cm and subdivided into three equal compartments with solid stainless steel walls to allow for triplicate testing per sampling period. Pebbles having a grain size of 4 - 15 mm were collected from the Isar River and used to fill the substratum cages which were finally placed in the middle of Flume A. This size fraction was selected as it approximated the D50 range typical in the Isar River (7.5 - 9.5 mm). Care had to be taken as some of the pebbles were smaller than the mesh size of the walls. The remaining sections in the flume before and after the substratum cages were filled-in with loose pebbles so as to maintain a constant bed depth over the length of the flume.

For large-scale Flume B, thirteen substratum cages were constructed to facilitate sampling of the bed sediments. The cages were again made of stainless steel, but measured 30 cm wide, 15 cm long, and 3 cm deep. The sides of the cages were made of stainless steel (mesh size of 1 cm) and the cage volume was again divided into three equal compartments by solid stainless steel walls to permit for triplicate testing per sampling period. Pebbles were collected from the Isar River and were sieved to obtain a size fraction between 15 and 30 mm. This fraction was slightly larger than D50 of 7.5 - 9.5 mm, however it was important to have stones large enough so they would not pass through the 1-cm wide mesh walls. The cages were filled with these stones and then placed in the middle of the flume. The rest of the flume bed was covered with

stones from the Isar River.

2.2.5 Lab-scale column reactor (Set-up C)

Two open, cylindrical batch reactors (FIGURE 2.4), 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 as well as the influence of turbidity on UV light penetration.

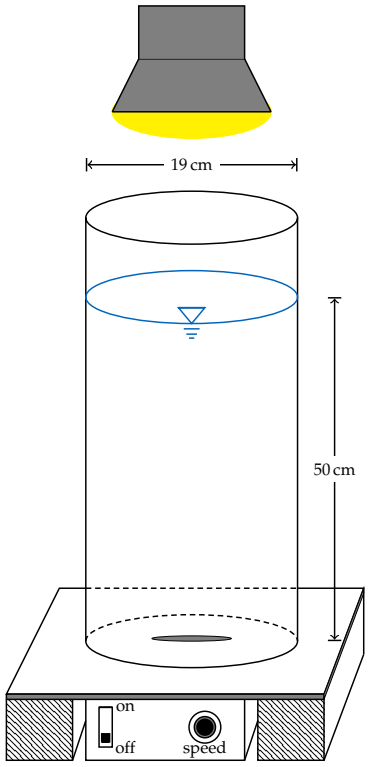


FIGURE 2.4: Column reactor outfitted with a borosilicate glass bottom and placed on top of a magnetic stirrer. UV sunlamp positioned above the 50 cm-deep water column to obtain an $I_{290-390nm}$ of 8.0 W m^{-2} at the water surface.

The bottom of the column was attached to a borosilicate glass plate to allow UV measurements to be made at a water depth of 50 cm. An artificial sunlight spectrum with a light intensity corresponding to the annual mean radiation in Germany ($I_{290-390nm} = 8.0\,W\,m^{-2}$) was generated using one overhead lamp per reactor column (Ultra-Vitalux, Osram, Germany). The column reactors were outfitted with water-chilled cooling jackets to maintain a water temperature of 14 °C. To promote mixing, the columns were placed on top of magnetic stirrers. Although impellers would have provided a more uniform mixing, their presence in the columns would have hindered uniform UV penetration.

2.3 Fate and transport of FIB and particles of wastewater origin in a lab-scale flume system mimicking an oligotrophic river

In lab-scale Flume A, three sets of experiments were conducted in the dark to investigate the fate and transport of FIB and other particles of wastewater origin in the water column and benthic biofilm of an oligotrophic river following a CSO. The benthic biofilm was allowed to develop for approximately six weeks, after which experiments could be conducted. To simulate such a CSO event, 1.5 to 2 l of municipal wastewater were added to Flume A (SECTION 2.2.2) which had been switched from fed-batch mode to recirculation mode. A ratio of wastewater to Isar River water of approximately 1:14 was always used, irrespective of the type of experiment, as the resulting FIB concentrations in the water column were representative of those resulting from CSO events. Details regarding the three groups of experiments are provided in the following and results are presented in CHAPTER 3.

2.3.1 Culture-based experiments investigating fate of FIB in the water column (Experiments $AI_{a,b,c}$)

In the first group of experiments in lab-scale Flume A, the focus was to determine removal rate coefficients of *E. coli* and enterococci in the water column following a CSO. Before wastewater was added to the flume to simulate such a CSO, one 25 ml wastewater sample and flume water sample were taken in two 50 ml, sterilized glass bottles to test for *E. coli*, enterococci, and COD. Thereafter, wastewater was added to the flume and sampling occurred after approximately 1, 2, 5, 12, 24, and 48 h. At each time interval, three 25 ml water samples were collected in individual 50 ml, sterilized glass bottles for *E. coli*, enterococci, and COD analyses. At the end of the experiment, a 2000 ml water sample was taken in a 2000 ml PP bottle to determine the TSS concentration. Due to the limited volume of Flume A and low turbidity, it was not possible to more frequently test TSS levels.

2.3.2 Culture-based experiments investigating deposition and attachment of FIB onto the benthic biofilm (Experiments $AII_{a,b,c}$)

In a second set of experiments conducted in lab-scale Flume A, the deposition and attachment of FIB to the benthic biofilm was investigated. Similar as in SECTION 2.3.1, before a CSO was simulated, 25 ml wastewater and flume water samples were collected in 50 ml, sterilized glass bottles to test for *E. coli*, enterococci, and COD. Additionally, three tiles were also removed at this time in order to analyze the biofilm for back-

ground *E. coli* and enterococci concentrations. The three tiles were carefully placed in three 50 ml sterilized, PP centrifuge tubes that had been pre-filled with 20 ml of phosphate buffered saline (PBS). Immediately after the sampling, three new tiles were inserted into the resulting gap, the flume was covered to ensure dark conditions, and the CSO was simulated by adding wastewater. After 24 h, the water in the flume was exchanged with fresh Isar River water after which three tiles were again removed and carefully placed in three centrifuge tubes pre-filled with 20 ml PBS. Based on the difference in FIB concentration before the CSO and after 24 h, the degree of FIB deposition and attachment to the benthic biofilm was determined.

2.3.3 Investigating the transport of wastewater particles in the water column and attachment to benthic biofilm with epifluorescence and confocal laser scanning microscopy (Experiments $\mathbf{AIII_{a,b,c}}$)

Identical experiments as described in SECTION 2.3.1 were conducted, whereby the aim was to detect fluorescently-labeled wastewater cells rather than *E. coli* and enterococci. The stain carboxyfluorescein diacetate, succinimidyl ester (CFDA; Vybrant® CFDA SE Cell Tracer Kit, Life Technolgies, USA) was selected for these experiments as it has been shown to label cells without jeopardizing cell viability or altering cell adhesion properties, while retaining a high degree of fluorescence for upwards of three weeks (Fuller et al., 2000).

Prior to the start of an experiment, 1.5 to 2 l of wastewater were stained with CFDA. To do so, a modified protocol was adapted from Fuller et al. (2000) and Augspurger et al. (2010). Firstly, the wastewater was equally divided between eight 250 ml PE centrifuge bottles and centrifuged for 20 min at 10 °C and 6000 rpm. Each of the eight pellets was subsequently resuspended in 20 ml of sterilized PBS; to promote resuspension, the PE bottles were placed in an ultrasonic bath (Sonorex Super RK514 BH, Bandelin; 35 kHz) for 10 min. Thereafter either 37.5 or 50 µl of a 10 mM stock solution of CFDA, depending on whether 1.5 or 2 l of wastewater was used, were added to each of the eight pellets. Based on the cell concentration, a target CFDA concentration of 1.46 mg l^{-1} was set according to Fuller et al. (2000). The eight bottles were wrapped in aluminum foil to avoid photobleaching and mixed overnight with the help of an overhead shaker.

The following morning, after approximately 12 h, the bottles were again centrifuged for 20 min at 10 °C and 6000 rpm. The resulting pellets were resuspended in sterilized PBS. To verify that the staining was successful, a 50 µl sample was diluted in 2 ml PBS and vacuum filtered over a polycarbonate filter having a pore size of 0.2 µm (Millipore Corp., Massachusetts, USA) and counterstained with 4',6-diamidino-2-phenylindole (DAPI). To confirm that the ratio of CFDA:DAPI stained cells was above 90 %, the filters were analyzed with an epifluorescence microscope.

Following a positive confirmation that CFDA staining was successful, the fluorescently-labeled wastewater was added to lab-scale Flume A to simulate a CSO. The flume was then covered to avoid exposure to light which could have led to photobleaching of the stained cells. Following the addition of stained wastewater to the system, 80 ml water samples were taken after approximately 0.5, 2, 5, 8, and 24 h. Additionally, one row of tiles was removed after roughly 0.5, 5, and 24 h for biofilm analysis. To assure that the hydrodynamic conditions remained more or less constant in the flume, tiles were firstly removed from the furthest position downstream. Thereafter, samples were taken in the upstream direction. When a row of tiles was removed, new tiles were then used to fill the resulting empty space.

2.4 Persistence of FIB in sediment of an oligotrophic river: Comparing behavior in lab-scale and large-scale flumes

Experiments were conducted in both Flumes A and B to gain a closer understanding of the potential for riverbed sediments to harbor *E. coli* and enterococci in an oligotrophic river. In both flume systems, experiments were performed with ceramic tiles and substratum cages (SECTION 2.2.4) to more closely evaluate the influence of sediment thickness on FIB persistence. Moreover, by using large-scale Flume B, the impact of a realistic bed shear stress on FIB persistence in bed sediments could be evaluated. Before experiments were started, the flumes were firstly operated for approximately six weeks to allow the benthic biofilm and sediment to fully develop. The experimental procedures used for both flumes will be described in the following. A discussion of the results is provided in CHAPTER 4.

2.4.1 Experiments in lab-scale Flume A (Experiments $AIV_{a,b}$ and AV)

Immediately before an experiment, the flume bed and water column were sampled and tested for background *E. coli* and enterococci concentrations. Thereafter, the UV lamps were switched off and the flume was covered. All experiments in Flume A were conducted in the dark to more closely mimic natural systems where light penetration is limited at deeper water depths. In order to ensure high initial FIB concentrations in the biofilm/sediment, the flume system was inoculated for 24 h with a 1:10 mixture of municipal wastewater and river water in recirculation mode. The following day, the walls of the flume were gently washed and the water was exchanged to ensure removal of all FIB not attached to the flume bed. At this point, samples were taken to determine the initial FIB concentrations in the flume bed and the system was switched to fed-batch mode operated with Isar River water. The volume of the system was exchanged approximately four times per day.

At each predefined time interval, the flume bed (cages or tiles) was sampled and one 25 ml water sample for FIB and COD testing (50 ml sterilized PP centrifuge tube) was collected. Due to the limited volume of the system and low turbidity (pre-sieved 100 µm), water samples for TSS were taken only at the start and end of an experiment (2000 ml PP bottle). Flume bed sampling occurred as follows: depending on the experiment, either one substratum cage or one row of three tiles was removed and any excess water was allowed to drain off for 10 s. The three tiles were placed in three centrifuge tubes that had been pre-filled with 20 ml of PBS. The contents of the three individual compartments of the substratum cage were transferred to three 250 ml sterilized glass bottles filled with 50 ml PBS.

Before the biofilm/sediment samples could be analyzed for culturable *E. coli* and enterococci, a preparation step was required for the tiles and substratum cages as outlined in SECTION 2.7.1 and SECTION 2.7.2, respectively. The substratum cage samples were additionally analyzed with qPCR according to the methods reported in SECTION 2.7.5 and SECTION 2.8.3.

2.4.2 Experiments in large-scale Flume B (Experiment BIII)

The experimental design used in Flume B was nearly identical to that described in SECTION 2.4.1, however a 1:1 mixture of municipal wastewater and river water was used for the inoculation. The inoculation did not occur in flow-through mode, but rather the inflow and outflow were stopped. This was done to ensure that initial FIB concentrations in the substratum would be comparable to the lab-scale flume. Moreover, the flume was not covered during the experiment, but rather was exposed to ambient sunlight. However, as the flume width (50 cm) was comparatively narrow with respect to the water depth (50 cm), UV light inactivation of sediment-associated FIB was considered to be negligible. Finally, the experiments were conducted in flow-through mode and not as a fed-batch system like Flume A due to the logistical problem of recirculating such a large volume of water for an extended period of time. Therefore the system could not be controlled as closely as the lab-scale flume, however the large-scale system more accurately depicts natural conditions.

At each sampling interval, one cage or one row of six tiles was removed in addition to a 100 ml water sample for COD (100 ml PP bottle) and 2000 ml for TSS (2000 ml PP bottle). Flume bed samples were handled as previously described in SECTION 2.4.1, except the contents of the cages were transferred to three individual 1 l sterilized glass bottles pre-filled with 400 ml PBS. The three pairs of tiles were placed in three centrifuge tubes that had been pre-filled with 20 ml of PBS. Here again, the biofilm/sediment samples required preparation before MPN and qPCR analyses could be performed. In SECTION 2.7.1, SECTION 2.7.3, and SECTION 2.7.5 detailed descriptions of the preparation methods are provided.

2.5 Influence of resuspension on the fate of FIB in large-scale flumes mimicking an oligotrophic river

A total of four experiments were conducted in large-scale Flume B to investigate the removal of FIB from the water column in a large-scale flume system mimicking the Isar River. The experiments to examine the effects of CSOs were subdivided into two phases. The first two experiments considered the removal of FIB from the water column over time at hydraulic conditions comparable to those in the Isar River. The final two experiments examined the impact of a sudden increase in discharge (bed shear stress) on FIB concentrations in the bulk phase. The results of these experiments are discussed in CHAPTER 5.

Prior to the start of an experiment, the flume was run for approximately six weeks in flow-through mode to ensure proper development of the benthic zone. Immediately before an experiment was started, the flume was then switched into recirculation mode. At this point, both water samples and individual stone samples were taken to provide more information regarding the background TOC, COD, TSS, and FIB concentrations. More information regarding sampling is provided in the following.

2.5.1 Standard removal experiments (Experiments $BI_{a,b}$)

The two standard removal experiments were conducted at a discharge of $0.2\,m^3\,s^{-1}$ which is equivalent to a flow velocity of $0.8\,m\,s^{-1}$. As a reference, water in the Isar River typically flows between 0.5 and $2.0\,m\,s^{-1}$. To simulate a CSO, $1\,m^3$ of municipal wastewater was added to the collection basin of Flume B operated in recirculation mode. This resulted in a 1:14 dilution of wastewater in Obernach River water. Following wastewater addition, 30 min were allowed prior to sampling to ensure adequate mixing.

Sampling always occurred in the middle of the flume (downstream length of 6 m), at three different water depths (top, middle, bottom). At each predefined time interval, the following water samples were taken: one 2000 ml for total suspended solids (1000 ml PP bottle), three 40 ml for *E. coli* and enterococci enumeration (50 ml sterilized centrifuge tubes), and one 100 ml for COD and TOC (100 ml PP bottle). Moreover, three stones were removed at each sampling interval from the bed for FIB enumeration. The stones were transported in individual 250 ml PP centrifuge bottles filled with 50 ml of PBS.

2.5.2 Resuspension experiments(Experiments $BII_{a,b}$)

For the final two experiments where the effects of resuspension were to be examined, the discharge in the flume was set at a lower discharge of $0.1\,m^3\,s^{-1}$ during the six-week cultivation period. At the start of the experiment, the flume was again switched to recirculation mode and wastewater was added to the collection basin to simulate a CSO. After a certain length of time, the discharge was increased at once to approximately $0.2\,m^3s^{-1}$ to induce resuspension of bed sediments. Thereafter, samples were collected in the same manner as described in SECTION 2.5.1.

For water samples where size exclusion chromatography coupled with dissolved organic carbon detection analyses were performed, collected water samples were gravity separated in an Imhoff cone for 2 h. Thereafter, samples of the settled sediment and overlying water were collected in 100 ml glass bottles and stored at 4 °C until analyzed (within 48 h).

2.6 Effect of particle association on UV inactivation of FIB in urban rivers

In an attempt to more accurately understand the influence of particle attachment and size on UV inactivation of FIB in river water following a CSO, three separate investigations were made. Firstly, the influence of varying TSS concentrations on UV light penetration was determined. Secondly, the range of particle sizes found in municipal wastewater and the association of *E. coli* and enterococci with them were measured. Finally, with this information a series of batch experiments were conducted in river water to determine UV inactivation rates of FIB associated with the different size fractions of wastewater. The experimental methods used are explained in the following and the results are described in CHAPTER 6.

2.6.1 Impact of TSS on UV attenuation (Experiment CI)

In an attempt to relate UV light attenuation with the TSS concentration and water depth, a series of measurements were made with water from the Obernach River in a lab-scale column reactor (FIGURE 2.4). Heavily TSS-laden water was collected from large-scale Flume B being operated in flow-through mode by scraping the bottom with a shovel and pumping the turbid water into two 100 l storage tanks. The TSS concentration of the water was immediately determined and based on this background concentration, dilutions were made with tap water to approximately achieve 10, 20, 30, 40, and $50\,mg\,l^{-1}$ TSS in the column reactor. For these five different concentrations, the UV intensity ($I_{290-390nm}$) at the bottom of the water column was determined for the

following water depths: 10, 25, and 50 cm.

Vertical attenuation coefficients in water were calculated for each TSS concentration using Beer's Law:

$$I_z = I_0 e^{-k_{att} z}\,, \tag{2.6}$$

where I_z is the irradiance in W m^{-2} at depth z in m, I_0 is the irradiance at the water surface, and k_{att} is the vertical attenuation coefficient (m^{-1}).

2.6.2 Particle size distribution and microbial fractionation of municipal wastewater (Experiment CII)

To gain more insight into the occurrence of specific particle sizes in municipal wastewater from the Garching treatment plant, on four different days, four 250 ml samples were collected in 250 ml PP bottles and stored at 4 °C until analysis was performed. Particle size distribution measurements 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 *E. coli* and enterococci. For this, 5 l of wastewater were separated into fractions by sieving through the following mesh sizes: 1000, 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 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 concentrations of the different size fractions were determined.

2.6.3 Experiments to investigate UV inactivation rates (Experiments CIII$_{a,b,c}$)

Two open cylindrical batch reactors (see FIGURE 2.4), were used to investigate the influence of particle association on inactivation of FIB in urban river water. Based on the results of the microbial fractionation of wastewater (SECTION 2.6.2 and SECTION 6.2.2), UV inactivation for the following fractions was investigated: $d_p \leq 12\,\mu m$, $12 < d_p \leq 63\,\mu m$, and $d_p > 1000\,\mu m$, where d_p represents the particle diameter. The two smallest fractions $d_p \leq 12\,\mu m$ and $12 < d_p \leq 63\,\mu m$ were chosen as they were found to have the highest FIB loading associated with them. Additionally, it was deemed relevant to investigate larger particles sizes ($d_p > 1000\,\mu m$) as they are known

to increase shielding and protection of attached FIB.

The same-sized sieves/filters as described in SECTION 2.6.2 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 $d_p > 1000\,\mu m$ was comparatively low, it was necessary to sieve 100 l of wastewater. For $12 < d_p \leq 63\,\mu m$, 2.5 l of wastewater were sieved and filtered and for $d_p \leq 12\,\mu m$ we sieved 2.5 l of wastewater and collected 300 ml which was subsequently filtered. The 300 ml filtrate from the 12 µm filter was subsequently centrifuged for 15 min at 6000 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 1 l of autoclaved water from the Isar River and added to one reactor. Mixing of the reactor contents was achieved with a magnetic stirrer. The experiment began when the UV lamp ($8\,W\,m^{-2}$ at water surface) 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. Additionally, at the end of each experiment a 2 l water sample was collected to determine the TSS concentration.

2.7 Sample preparation

2.7.1 Biofilm on ceramic tiles

To test for *E. coli* and enterococci concentrations in the benthic biofilm covering the ceramic tiles in Flumes A and B, the biofilm on each tile needed to be removed. Firstly, with the tile still submerged in 20 ml PBS, the biofilm was scraped off with a new toothbrush. Subsequently, the toothbrush and tile were rinsed with a total of 10 ml sterile PBS. The centrifuge tube containing 30 ml PBS and biofilm was then centrifuged for 20 min at 6000 rpm and 10 °C. In order to concentrate the sample, 15 ml of the supernatant were removed and the remaining 15 ml were homogenized with a teflon piston on ice for 5 min at 2500 RPM (RW 20 DZM, IKA, Staufen, Germany). This 15 ml concentrated sample was then used to test for FIB concentrations. The concentration in the biofilm ($C_{FIB,F}$) is reported in MPN $g_{DM}{}^{-1}$. Because the resulting 15 ml solution was needed entirely for MPN testing, separate tiles were used to determine the average dry matter content ($DM_{sediment}$) of the biofilm. The same preparation procedure as already described was again used.

2.7.2 Biofilm/sediment from substratum cages: Lab-scale Flume A

To separate attached FIB from the stones, the 250 ml glass bottle containing 50 ml PBS and biofilm-covered stones was placed in an ultrasonic bath (35 kHz) for 20 min. Af-

terwards, the solution was transferred to a new sterile 250 ml glass bottle and the stones were retained in the original glass bottle. The stones were then rinsed with 30 ml PBS in the original glass bottle. This solution was subsequently added to the new bottle containing 50 ml PBS and biofilm/sediment. The resulting 80 ml solution of PBS and biofilm/sediment were used for determining FIB concentrations ($C_{FIB,S}$) and $DM_{sediment}$. Values of $DM_{sediment}$ are reported with respect to the substratum cage's surface area ($g_{DM}\,m^{-2}$); $C_{FIB,S}$ is reported as MPN $g_{DM}{}^{-1}$ or copies $g_{DM}{}^{-1}$.

2.7.3 Biofilm/sediment from substratum cages: Large-scale Flume B

To remove FIB from the stones, the following steps were taken. Initially, the 1 l glass bottle filled with 400 ml PBS, stones, and sediment were placed in an ultrasonic bath (35 kHz) for 20 min. Thereafter, the resulting solution in the bottle was transferred to a sterilized 1 l glass bottle via a sterilized stainless steel funnel/sieve system (mesh size 2.5 mm). Sediment and water could pass through the funnel/sieve set-up, however the stones were retained. The funnel/sieve were then rinsed with 100 ml sterile PBS. This solution was used to test for FIB concentrations and $DM_{sediment}$. The FIB concentration in the biofilm/sediment is reported in MPN $g_{DM}{}^{-1}$ or copies $g_{DM}{}^{-1}$. $DM_{sediment}$ concentrations are again related to the substratum cage's surface area ($g_{DM}\,m^{-2}$).

2.7.4 Biofilm from individual stones: Large-scale Flume B

To investigate the concentrations of *E. coli* and enterococci on individual stones in the large-scale flume (SECTION 2.5.1), the biofilm needed to be detached. Firstly, with the stone still submerged in the 50 ml of PBS, the biofilm was scraped away with a new toothbrush. Thereafter, the toothbrush and stone were rinsed off with 20 ml sterile PBS and the stone was set aside. The 70 ml PBS/biofilm solution was subsequently centrifuged for 20 min at 6000 rpm and 10 °C. In order to concentrate the sample, 60 ml of the supernatant were carefully removed. The remaining 10 ml solution was homogenized with a teflon piston on ice for 5 min and then used to test for FIB concentrations.

The FIB concentrations were subsequently related to the surface areas of the stones. To do this, each stone was carefully wrapped in aluminum foil, avoiding any overlapping or wrinkling. The aluminum foil was then weighed and compared to the mass of a 2 cm x 2 cm reference piece of foil to determine the surface area.

2.7.5 Sample filtration for qPCR

In order to prepare samples for qPCR analyses, the method as described in Reischer et al. (2008) was adapted accordingly. For each sample, a volume of 10 ml (lab-scale

Flume A) or 25 ml (large-scale Flume B) was filtered through 47-mm diameter, 0.2-µm pore size polycarbonate filters (catalog # GTBP04700, Millipore Corp., Eschborn, Germany) and subsequently inserted into individual, 1.5 ml PCR-clean, DNA LoBind tubes (catalog # 0030108051, Eppendorf, Hamburg Germany). All samples were stored at 4 °C prior to filtering, which occurred within 24 h. Thereafter, all filters were stored at -20 °C before extraction.

DNA was recovered from the filters through a phenol-chloroform extraction. 400 µl chloroform isoamyl alcohol, 400 µl CTAB buffer, 400 µl phenol, and the filters were added to a tube containing glass beads (catalog # 116914050, MP Biomedicals, France). Bead milling subsequently occurred in a FastPrep®-24 System (MP Biomedicals, France) at a speed of 6.0 for 30 s, after which the tubes were allowed to cool to room temperature in an ice water bath. The tubes were subsequently centrifuged at 16,100 × g for 5 min at room temperature to pellet the glass beads and debris. The resulting supernatants were transferred to new 1.5 ml tubes to which 270 µl of isopropanol was added and vortexed. Thereafter, the tubes were centrifuged at 16,100 × g for 15 min at room temperature. The resulting supernatant was then discarded and 1 ml of chilled 70% ethanol was added. The tubes were once again centrifuged at 21,900 × g for 5 min at 4 °C. The supernatant was removed with a water-jet vacuum pump fixed with an elongated glass Pasteur pipette. The tubes were left open, but covered, in the fume hood overnight in order to dry. Finally, 20 µl of PCR-clean water (Qiagen, Hilden, Germany) was added to the DNA and stored at -20 °C.

2.8 Analyses performed

2.8.1 Physico-chemical Parameters

Total and volatile suspended solids (TSS, VSS) concentrations were determined according to German standard methods DIN 38414 (2005; S10) and 38409 (1987; H2), respectively. Wet-chemical analysis (Hach Lange, LCK 414, Dusseldorf, Germany) was used to determine COD concentrations. TOC was measured according to the standard method DIN EN 1484 using a highTOCII (Elementar Analysesysteme GmbH, Hanau, Germany). The dissolved oxygen concentration (DO), water temperature, and pH were measured with the Multi 340i meter (WTW, Weilheim, Germany). The photodetector PeakTech 5085 (PeakTech, Ahrensburg, Germany) was used to determine the ultraviolet intensity (λ = 290 - 390 nm). A laser diffraction particle size distribution analyzer (*Partica* LA-950, Horiba Instruments, USA) located at the Bundeswehr University (Neubiberg, Germany) was used for particle size measurements. Following collection, TOC and COD samples were stored at -20 °C until analysis. Samples for TSS and VSS analyses were refrigerated at 4 °C and analyzed within 24 h of sampling.

Size exclusions chromatography with dissolved organic carbon detection (SEC-DOC) was performed by Matthias Weber of the Karlsruhe Institute of Technology to characterize the DOC associated with both suspended solids and river water. The TOC was determined with a TOC-V_{CSN} Analyzer (Shimadzu Scientific Instruments) and the SEC-UVA/OCD-system as described in Huber and Frimmel (1991) was used. The same method as described in Huber and Frimmel (1991) was implemented.

2.8.2 Culture-based method to determine *E. coli* and enterococci concentrations

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, 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 ($\lambda = 360\,\mathrm{nm}$) 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 for water samples when using four serial dilutions is $3.8 \times 10^{-1}\,\mathrm{MPN\,ml^{-1}}$. As all FIB concentrations in the sediment/biofilm are reported with respect to a sample's $DM_{sediment}$, the detection limit was slightly different for each sample. When concentrations fell below the method detection limit, a uniform value of $50\,\mathrm{MPN\,g_{DM}}^{-1}$ was used. All samples for *E. coli* and enterococci analyses were refrigerated at 4 °C and analyzed within 24 h of sampling.

2.8.3 Molecular-based method to detect enterococci: qPCR

Reactions were prepared in 1.5 ml PCR-clean tubes (Eppendorf, Hamburg, Germany) by addition of the following components: 12.5 µl Quanti Tec Probe PCR Master Mix (catalog # 204363, Qiagen, Hilden, Germany); 1.5 µl (10 pM each) forward and reverse primer (Eurofins MWG Operon, Ebersberg, Germany); 0.75 µl TaqMan™ probe (Eurofins MWG Operon, Ebersberg, Germany); 1 µl 50 mM $MgCl_2$; 5.25 µl PCR-clean water; 2.5 µl DNA template. Reactions were monitored in a GeneAmp® 5700 Real-Time Cycler (Applied Biosystems). Thermal cycling conditions were adapted with minor modifications from (Haugland et al., 2005) in order to achieve a PCR amplification efficiency of 102 % and consisted of 15 min at 95 °C, 35 cycles of 15 s at 95 °C, and 2 min at 60 °C.

The PCR primer and hybridization probe sequences for the enterococci assay were adapted from Ludwig and Schleifer (2000) and consisted of: ECST748F: 5'-AGAAATTC-

CAAACGAACTTG for the forward primer; ENC854R: 5′-CAGTGCTCACCTCCATCATT for the reverse primer; and GPL813EQ: 5′-6FAM-TGGTTCTCTCCGAAATAGCTTTAGGGCTA-TAMRA for the probe, whereby FAM was the fluorophore and TAMRA the quencher. Three replicate reactions were performed per sample dilution. In total, six 10-fold serial dilutions of PCR standard, a no-template, and no-amplification control were performed for each plate.

2.8.4 Epifluorescence microscopy

To determine the concentration of stained cells in the water column at a given time, 2.5 ml of an 80 ml sample were vacuum filtered over a black, polycarbonate filter having a pore size of 0.2 µm (Millipore Corp., Eschborn, Germany). Filters were mounted on glass slides and viewed with an epifluorescence microscope (Aristoplan, Leitz, Germany) equipped with a 100× oil immersion lens (numerical aperture 1.3). For each filter, cell counts were obtained for ten microscopic fields from which a mean value was derived. These cell counts were converted to concentrations (C_{cells}) with the following formula:

$$C_{cells} = \left(\frac{A_{filter}}{A_{MF}} \right) X_{MF} V \,, \tag{2.7}$$

where A_{filter} is the filter area (346 mm^2), A_{MF} is the microscopic field area (0.025 mm^2), X_{MF} is the average cell number per microscopic field, and V is the dilution factor (ml^{-1}).

Where the attachment of CFDA-stained cells to benthic biofilms (SECTION 2.3.3) was investigated, it was necessary to compare the volume of stained wastewater cells added to the flume system with the volume of CFDA-signal detected in the biofilm with CLSM. To estimate the initial volume of stained cells added to the system, a 50 µl stained wastewater sample was added to 2 ml of PBS and was subsequently vacuum filtered over a polycarbonate filter. The filter was subsequently viewed with the epifluorescence microscope (100× oil immersion lens; numerical aperture 1.3). 15 microscopic fields (86.67 µm × 68.67 µm) were imaged (AxioCam, b/w, Carl Zeiss MicroImaging GmbH, Jena, Germany) and the area of stained cells was calculated with the image processing package Fiji (freeware; Schindelin et al., 2012). Firstly, the images were manually thresholded so that only the pixels of interest were accounted for. Pixels with values less than the set threshold were thus reassigned to the background. Based on the pixel counts and knowing the dimensions of one pixel, the overall surface area of CFDA-stained cells in the wastewater was determined with Equation 2.7. This surface area needed to be converted into a volume to allow for a comparison with voxel counts from CSLM. Thus, it was necessary to assume that the cells formed a monolayer on the filter with a thickness of 1 µm.

2.8.5 CLSM: Image acquisition and analysis

Image stacks were acquired with an upright laser scanning microscope (LSM 510 META, Carl Zeiss MicroImaging GmbH, Jena, Germany) controlled by the AIM software package (version 3.2, Carl Zeiss MicroImaging GmbH, Jena, Germany). The excitation of CFDA-stained wastewater particles was achieved with an argon laser which emitted light at a wavelength of 488 nm; emission signals were collected with a band filter between 505 and 545 nm. The natural autofluorescence of cyanobacteria and algae present in the biofilm was excited with a helium-neon laser at 633 nm and emission signals were collected from 650 to 720 nm. Reflection signal from the tile and inorganic constituents of the biofilm was excited at 488 nm; emission signals were collected between 475 and 490 nm. Samples were scanned with a 40× water immersible lens (numerical aperture 0.8).

The main focus of these CLSM analyses was to investigate attachment of CFDA-stained wastewater particles to the base biofilm. Cyanobacteria and algae were abundant in the base biofilm and therefore, provided enough structural information without the need for additional information regarding extracellular polymeric substances and native bacteria. The pinhole was adjusted for each channel so that all channels were acquired with an optical slice thickness of 0.78 µm. For each of the three tile samples removed during sampling, five random regions were imaged, corresponding to a scanned surface area of 0.27 mm^2.

Digital image analysis of the acquired stacks was again performed with the image processing package Fiji. For visualization purposes, maximum intensity projections (MIP) were generated with this freeware. A MIP is a means to visualize three-dimensional information in two dimensions and allows for a qualitative assessment of an image. When the optical slices of an image are laid on top of each other (3D), only the pixels with the highest intensity are used to create the two-dimensional image. This concept is depicted in FIGURE 2.5.

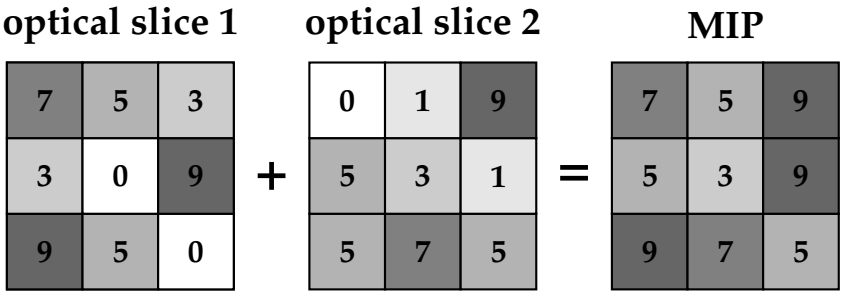


FIGURE 2.5: Schematic drawing of how a maximum intensity projection (MIP) is created from two optical slices. Adapted from Wagner (2011).

The tool JImageAnalyzer 1.1, which is based on Fiji, was used for signal quantification. As each image was individually adjusted during acquisition, image stacks

were also manually thresholded. To characterize the biofilms, the mean thickness and roughness coefficient were used. The mean biofilm thickness, $\overline{L}_F$, is the average distance from the top of the biomass to the substratum, or in this case the ceramic tile surface. The mean thickness can be determined by depth-coding an image stack and taking the average grayscale value. The roughness coefficient, R_a, can subsequently be calculated with the following equation:

$$R_a = \frac{1}{N} \cdot \sum_{i=1}^{N} \frac{|L_F(i) - \overline{L}_F|}{\overline{L}_F}, \tag{2.8}$$

where N is the number of thickness measurements and $L_f(i)$ is the thickness at a certain position $i(x, y)$ (Murga et al., 1995).

2.9 Overview of experiments performed

In order to assist the reader, an overview all experiments conducted is provided in TABLE 2.2.

TABLE 2.2: Overview of all experiments conducted in this work.

experiment	system	discharge ($l\,s^{-1}$)	duration (h)	$UV_{(290-390nm)}$ ($W\,m^{-2}$)	aim
$AI_{a,b,c}$	lab-scale flume	0.3	40, 49, 48	0	removal of FIB from water column
$AII_{a,b,c}$	lab-scale flume	0.3	24, 24, 24	0	deposition and attachment of FIB onto benthic zone
$AIII_{a,b,c}$	lab-scale flume	0.3	24, 24, 21	0	removal of CFDA-stained cells from water column and attachment onto benthic zone
$AIV_{a,b}$	lab-scale flume	0.3	97, 312	0	FIB persistence on biofilm-coated ceramic tiles
AV	lab-scale flume	0.3	643	0	FIB persistence in substratum cages
$BI_{a,b}$	large-scale flume	200	72, 69	3, 7	removal of FIB from water column and deposition onto benthic zone
$BII_{a,b}$	large-scale flume	100→ 200	69, 73	17, 9	removal of FIB from water column following resuspension event
BIII	large-scale flume	200	491	0	persistence of FIB in riverbed sediment
CI	column reactor	gently mixed	-	8	impact of TSS on UV attenuation
CII	-	-	-	-	particle size distribution of wastewater; association of FIB with different fractions
$CIII_{a,b,c}$	column reactor	gently mixed	48, 48, 49	8	effect of particle association on UV inactivation of FIB

Chapter 3

Fate and Transport of FIB and Particles of Wastewater Origin in a Lab-Scale Flume System Mimicking an Oligotrophic River

In order to investigate the persistence of FIB in an oligotrophic river following a CSO, a lab-scale flume system representing the Isar River was constructed (FIGURE 2.1). One series of experiments were designed to examine the behavior of *E. coli* and enterococci in the water column under dark conditions. A second set of experiments were conducted to explore the transport of wastewater particles from the water column to the benthic biofilm, again without the influence of light. Particle deposition was followed using epifluorescence and confocal laser scanning microscopy.

3.1 Physico-chemical characteristics during experiments

All flume experiments were conducted in the dark with Isar River water tempered at approximately 12 °C and having an average pH of 8.2. Both the TSS and COD concentrations measured in the water column were consistently below $10\,\mathrm{mg\,l^{-1}}$. The benthic biofilm that formed on the ceramic tiles had an average dry matter content of $43\,\mathrm{g_{DM}\,m^{-2}}$, whereby more than 95 % was organic.

3.2 Culture-based experiments investigating fate of FIB in the water column

A group of experiments as described in SECTION 2.3.1, were conducted to more closely understand the fate of *E. coli* and enterococci in the water column following a CSO. Specifically, the degree of deposition and attachment to the benthic biofilm was to be

determined. Additionally, attempts were made to understand the individual contributions of sedimentation and natural inactivation on FIB removal from the water column.

3.2.1 Fate of FIB in the water column (Experiments $AI_{a,b,c}$)

In FIGURE 3.1, the fate of FIB in the water column following a CSO is presented. The mean *E. coli* and enterococci concentrations over two days are depicted. Before each experiment, FIB concentrations in the water column were below the detection level of 3.8×10^{-1} MPN ml^{-1} and are thus, not shown. Following the addition of wastewater to the flume to simulate a CSO, initial ($t = 0$ h) *E. coli* and enterococci concentrations were approximately 2000 and 400 MPN ml^{-1}, respectively. For both types of FIB, similar removal was observed which could be described by first-order kinetics. The dark removal rate coefficients for *E. coli* (k_{ec}) and enterococci (k_{ent}) were 0.05 h^{-1} ($R^2 = 0.86$) and 0.06 h^{-1} ($R^2 = 0.97$), respectively.

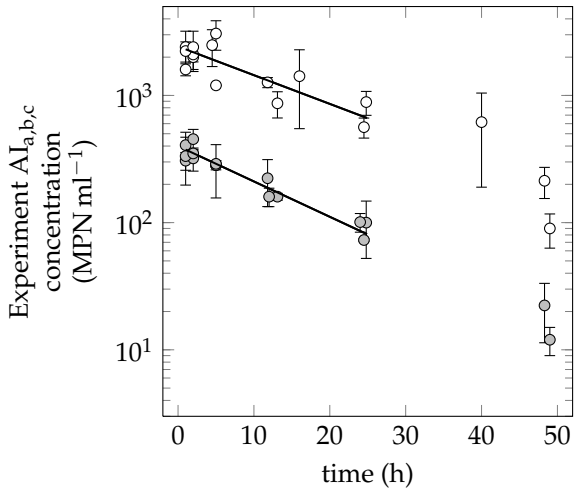


FIGURE 3.1: *E. coli* (white) and enterococci (gray) removal from the water column of lab-scale Flume A. For *E. coli* a first-order removal rate coefficient (k_{ec}) of 0.05 h^{-1} ($R^2 = 0.86$) was determined and for enterococci, $k_{ent} = 0.06$ h^{-1} ($R^2 = 0.97$).

Numerous studies have been conducted to explore the impact of various biotic and abiotic conditions such as temperature, solar irradiance, and predation on FIB persistence in the aquatic environment. Published removal coefficients range from 0.008 to 0.95 h^{-1} (Sinton et al., 2002; Menon et al., 2003; Chigbu et al., 2005; Servais et al., 2007; Schultz-Fademrecht et al., 2008; Mitch et al., 2010). Schultz-Fademrecht et al. (2008) conducted a similar set of flume experiments with the aim of more closely understanding the impact of UV light on FIB inactivation. Under dark conditions, the authors determined removal coefficients for *E. coli* and enterococci of 0.10 and 0.07 h^{-1}, respectively, which are in close agreement with those determined in the present work.

No appreciable difference in the removal rates of *E. coli* and enterococci was observed, however this perhaps is not surprising. In the presence of UV light, enterococci

have been shown to be more robust than *E. coli* (Harris et al., 1987; Sinton et al., 1994; Rincón and Pulgarin, 2004; Sinton et al., 2007; Schultz-Fademrecht et al., 2008). As the experiments in this study were conducted in the dark, UV light had no influence on removal rates. Moreover, as the water used in the flume was pre-filtered (100 µm), predation should not have been an important factor. The most likely cause of reduced FIB concentrations in the water column was natural inactivation due to the low nutrient levels and water temperature. Additionally, deposition out of the water column onto the benthic biofilm may have also contributed to the overall removal rates, which will be discussed in the following section.

3.2.2 Deposition of FIB onto the benthic biofilm (Experiments $AII_{a,b,c}$)

From the results presented in SECTION 3.2.1, the overall removal rate coefficient of FIB from the water column is understood. However, a differentiation between the individual contributions of deposition and natural inactivation cannot be made. Therefore, three experiments as described in SECTION 2.3.2 were conducted to clarify the impact of deposition on the overall removal rate coefficients. The results presented in TABLE 3.1 indicate that after 24 h, less than 10 % of the culturable FIB added to the system with the wastewater were found again in the benthic biofilm. There was most likely some degree of inactivation in both the water column and benthic biofilm during the first 24 h, which indicates that the actual degree of deposition may have been slightly higher than what is reported in TABLE 3.1.

TABLE 3.1: Fraction of *E. coli* and enterococci in the added wastewater that was then found in the benthic biofilm after 24 h.

experiment	*E. coli* (%)	enterococci (%)
AII_a	1.2	1.3
AII_b	7.9	4.3
AII_c	5.0	5.7

The theoretical amount of FIB expected in the biofilm after 24 h was determined based on the known amount of FIB initially added to the system and $k_{ec,ent} = 0.05$ and $0.06\,h^{-1}$ as determined in SECTION 3.2.1. Based on this calculation, approximately 30 % of *E. coli* and enterococci added to the system should have been found in the biofilm after 24 h, which is considerably higher than what was actually seen (TABLE 3.1). These theoretical values were calculated by making the assumption that all FIB removed from the water column subsequently attached to the benthic biofilm. This most likely was not the case as attachment to the flume setup (walls, tubes, pump) is inevitable. Moreover, the calculation assumed no loss in FIB culturability over time which given the

low water temperature and nutrient levels, is not necessarily plausible.

From FIGURE 3.1, it can be seen that within the first 24 h roughly 30 % of the FIB initially present in the water column had been removed. Assuming that deposition onto the biofilm contributed 5 % towards this FIB removal (TABLE 3.1), it becomes evident that approximately $\frac{5}{30}$ (17 %) of the overall FIB removal from the water column was due to deposition out of the water column and $\frac{25}{30}$ (83 %) resulted natural inactivation. With this knowledge, the overall FIB removal rate coefficients from the water column ($k_{FIB} = 0.05 - 0.06\,\mathrm{h}^{-1}$) were adjusted to $k_{D,FIB} = 0.01\,\mathrm{h}^{-1}$ to account for deposition and $k_{I,FIB} = 0.04\,\mathrm{h}^{-1}$ for natural inactivation. In a study by Milne et al. (1986), deposition rates of fecal coliforms were determined in lab-scale experiments using estuary water with varying suspended solids concentrations and no flow. At low TSS concentrations ($< 20\,\mathrm{mg\,l}^{-1}$), a deposition coefficient of approximately $0.01\,\mathrm{h}^{-1}$ was determined. Although it is difficult to compare results from the two different setups, the adjusted $k_{D,FIB} = 0.01\,\mathrm{h}^{-1}$ determined in this study appears to be in the correct range.

Several studies were found in literature which present settling velocities for specific particle fractions rather than microorganism-specific deposition coefficients (Auer and Niehaus, 1993; Canale et al., 1993; Cushing et al., 1993; Thomas et al., 2001; Battin et al., 2003; Jamieson et al., 2005a). For example, with a modeling technique Jamieson et al. (2005a) found that for particle sizes between 45 and 75 µm, settling velocities varied between $2 - 3 \times 10^{-5}\,\mathrm{m\,s}^{-1}$ in two rivers. Battin et al. (2003) performed a set of experiments in large-scale flumes (length 30 m; width 0.30 m; depth 0.30 m) at different flow velocities and found particle deposition velocities up to 250× faster for organic particles 53 - 106 µm in size ($3 - 8 \times 10^{-3}\,\mathrm{m\,s}^{-1}$). In this study, an attempt has been made to derive the FIB-specific deposition velocity, v_{dep}, with the following equation originally presented by Newbold et al. (1981):

$$v_{dep} = \frac{uz}{x}\,, \tag{3.1}$$

where x is the transport distance (m) of the particles. The transport distance can be determined with:

$$x = L \cdot \ln\left(\frac{C_0}{C_{24\,\mathrm{h}}}\right)\,, \tag{3.2}$$

where L represents the flume length and C the FIB concentrations at $t = 0$ and 24 h. As the flume system used in this study was operated in recirculation mode, L had to be adjusted accordingly. By knowing that the ratio of time required to pass the flume bed to the time required to pass through the entire flume system (tubing, recycle bucket) was 0.25, L was calculated with the following equation:

$$L = \frac{ut}{4}\,. \tag{3.3}$$

The deposition velocity calculated for *E. coli* and enterococci was found to be $5 \times 10^{-6}\,\mathrm{m\,s^{-1}}$, which is markedly lower than the rates determined by Battin et al. (2003) and Jamieson et al. (2005a). Both authors however based their deposition rates on measurements taken over a flume length or river stretch of 30 m or more. As the flume bed in the current work was only 1.2 m long, such an underestimation of the deposition velocity can be expected due to the frequent re-mixing that occurs in the recycle bucket after water exits the flume. Moreover, the authors were considering particle sizes between 45 and 106 µm. As will be shown in SECTION 6.2.2, more than 90 % of *E. coli* and 80 % of enterococci present in the wastewater used in this study are found to be associated with particle sizes $\leq 12\,\mu\mathrm{m}$. If deposition velocities are thus based on this fraction, slower rates are conceivable.

As it has been shown that deposition rates in natural waters are directly proportional to TSS concentrations (Milne et al., 1986), it should be stressed that the findings of the present study are based on river water having a low TSS concentration ($< 10\,\mathrm{mg\,l^{-1}}$).

3.3 Tracking of wastewater cells with microscopy techniques (Experiments $\mathrm{AIII_{a,b,c}}$)

To gain more insight into the transport of wastewater particles following a CSO, identical experiments as described in SECTION 3.2.1 were conducted except wastewater cells were stained with the dye CFDA. A more detailed description of the experimental method is provided in SECTION 2.3.3. By fluorescently labeling the cellular constituent of wastewater, detection was based solely on fluorescence. Therefore, all issues surrounding loss of viability and culturability were eliminated from detection.

3.3.1 Removal of wastewater cells from the water column

In an attempt to calculate a removal coefficient of the stained wastewater cells, water samples were collected and filtered. Finally, the stained cells retained on the filters were enumerated with an epifluorescence microscope as described in SECTION 2.8.4. As there was no influence of inactivation, the removal rate of wastewater cells from the water column was understood to be the deposition rate. In FIGURE 3.2, the mean concentrations of CFDA-stained wastewater cells in the water column throughout the first 24 h of four experiments are presented. The concentrations have been normalized to facilitate comparison between different experiments where varying amounts of raw wastewater were stained (1.5 - 2 l).

It can be seen that during the first 24 h, removal of CFDA-stained cells was characterized by first-order kinetics. From the experimental data, an average deposition

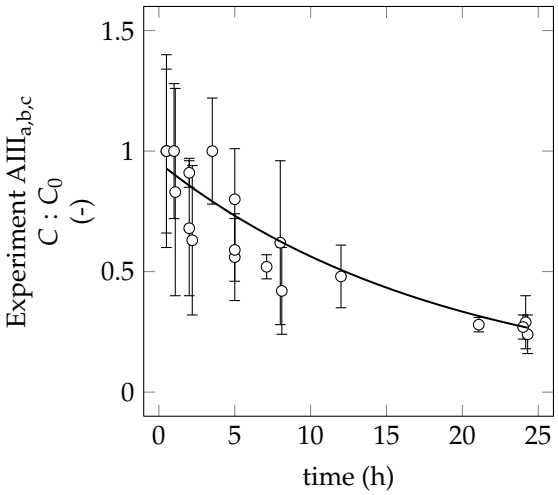


FIGURE 3.2: Normalized mean concentration of CFDA-stained wastewater cells in the water column of lab-scale Flume A. Trend line depicts first-order removal with a removal rate coefficient ($k_{D,CFDA}$) of 0.05 h^{-1} (R^2=0.88).

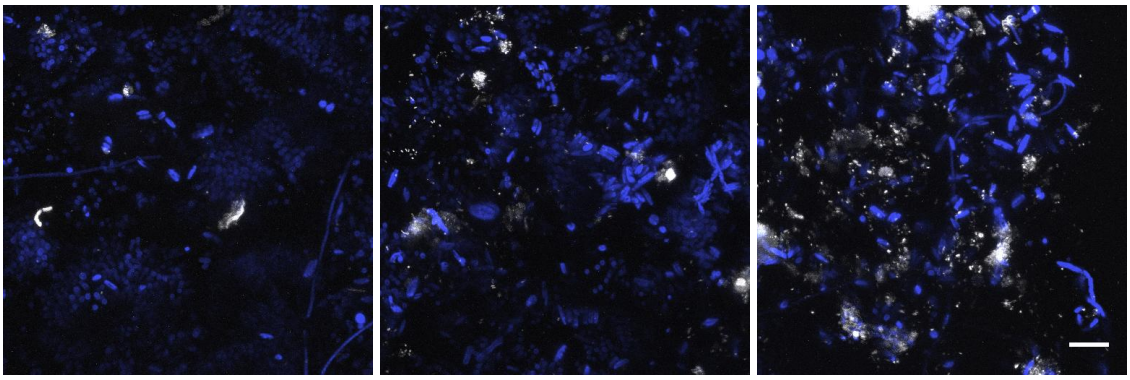
rate coefficient, $k_{D,CFDA}$, of 0.05 h^{-1} ($R^2 = 0.88$) was determined. In comparison to the approximate overall FIB removal rates determined in SECTION 3.2.1 ($k_{ec,ent} = 0.05 - 0.06\,h^{-1}$), the removal rate coefficient determined for stained bacteria is the same. This finding would indicate that nearly all FIB removal from the water column in lab-scale Flume A resulted from sedimentation/advective transport and that natural inactivation played little to no role.

A possible explanation for this discrepancy lies in the two different methods used and how particles were detected and recorded. For the epifluorescence technique, all stained cells were counted, irrespective of whether or not they were clumped together, particle associated, or freely-suspended. The FIB-specific microplate techniques based on the presence or absence of a specific enzyme, however do not distinguish between FIB that are freely suspended and those that are agglomerated. Larger particles and cell clusters have been shown to be removed faster from the water column than freely-suspended cells (Droppo, 2004; Searcy et al., 2005). The more rapid removal of these clusters would only have been registered by the microscopy technique and would not have been visible through MPN counts. Therefore, it is possible that the removal of larger particles during the first 24 h may have attributed to the more rapid removal rates detected with the microscopy technique. Additionally, not only were *E. coli* and enterococci stained by CFDA, but all cells present in the wastewater. The $k_{D,CFDA}$ presented here most likely provides an overestimation of FIB removal due to deposition.

3.3.2 Attachment of wastewater cells to the benthic biofilm

In addition to analyzing the water column, confocal laser scanning microscopy as described in SECTION 2.8.5, was utilized to investigate the attachment of wastewater cells

onto the benthic biofilm. The deeper such cells are within a biofilm, the more protected they are from adverse hydrodynamic conditions, predators/grazers, UV light and thus, chances of survival increase. Therefore, the initial aim of these experiments was to quantify where in the biofilm the stained wastewater cells/aggregates landed. In order to increase representativeness, five CLSM stacks were acquired per sample, equating to a scanned surface area of 0.27 mm^2. In the representative maximum intensity projections presented in FIGURE 3.3 for one experiment, it can be seen that as the experiment progressed there was an increase in the abundance of CFDA-stained wastewater cells (white signal) attached to the benthic biofilm.



(a) $t = 0.5\,h$ **(b)** $t = 5\,h$ **(c)** $t = 24\,h$

FIGURE 3.3: Maximum intensity projections depicting accumulation of CFDA-stained wastewater cells in the benthic biofilm over time during Experiment $AIII_c$. Blue represents the base biofilm (autofluorescence) and white the CFDA-stained cells. The scale bar has a length of 25 µm.

In an attempt to quantify this qualitative observation, all acquired image stacks from three parallel experiments were analyzed and the overall volume of CFDA-stained cells (V_F) per sample was calculated. The results were then related to the initial volume of CFDA-stained wastewater cells added to the system (V_{ww}) and are presented in FIGURE 3.4. The volume of stained cells in the wastewater was determined with the method described in SECTION 2.8.4. It needs to be emphasized that only two-dimensional images were acquired with the epifluorescence microscope. Thus, it was necessary to assume that cells retained on the polycarbonate filter comprised a monolayer with a thickness of 1 µm for comparison with the three-dimensional images acquired with CLSM.

From FIGURE 3.4, it can be seen that over a period of 24 h, an accumulation of CFDA-stained cells in the biofilm occurred which could be described by the function: $\log(V_F : V_{ww}) = 0.71 \log(t) + \log(0.38)$ $(R^2 = 0.92)$. On average, 3.6 % of the stained wastewater particles initially added to the system were detected in the biofilm after 1 d. This degree of attachment is quite similar to that obtained for *E. coli* and enterococci as previously highlighted in TABLE 3.1.

The theoretical amount of fluorescently-labeled cells that should have been found

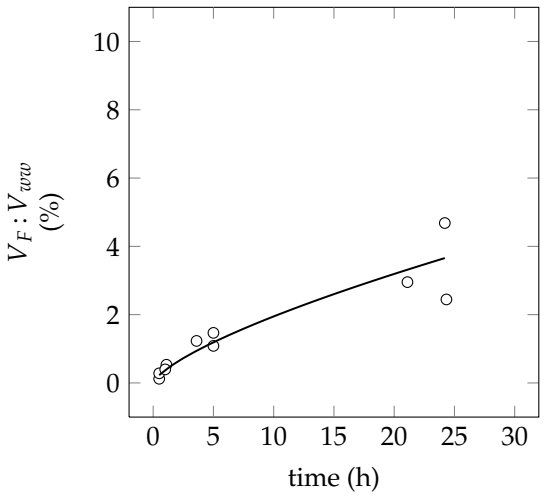


FIGURE 3.4: Accumulation of stained wastewater particles in the flume experiment as presented in SECTION 3.3. The increase in CFDA signal in the biofilm was described with the following function: $\log(V_F : V_{ww}) = 0.71 \log(t) + \log(0.38)$ ($R^2 = 0.92$), where V_F and V_{ww} represent the volumes of CFDA signal inside the benthic biofilm and of CFDA-stained wastewater cells added at $t = 0$ h, respectively.

in the biofilm after 24 h was again calculated by knowing the volume of CFDA-stained particles added to the system and $k_{D,CFDA} = 0.05\,\mathrm{h}^{-1}$ as determined in SECTION 3.3.1. After 24 h, on average 33 % of the stained cells added should have been recovered in the biofilm. This again is substantially higher than the actual value of 3.6 % which was determined with CLSM (FIGURE 3.4). Some portion of these losses are attributed to attachment of the stained cells to the flume setup. Further, the degree of attachment of CFDA-stained cells to the biofilm may have actually been higher than what was measured with CLSM due to laser penetration issues. Limited laser penetration in deep and/or dense biofilms is a well-known problem (Heydorn et al., 2000; Beyenal et al., 2004), which could have resulted in CFDA-stained cells located in deeper regions of the biofilm not being detected. Attempts were made to avoid photo-bleaching of the CFDA stain (scanning from top to bottom), however this is also an issue surrounding CLSM. Moreover, it was only possible to investigate a cross-sectional area of 0.27 mm^2 due to long image acquisition times (45 - 60 min). As the deposition of wastewater cells on the biofilm was not necessarily homogeneous, the acquired image stacks may not provide a representative account of particle attachment. Thus, it is arguable whether or not measurements made at the microscale can be extrapolated to accurately predict behavior at the mesoscale. In a study conducted by Wagner et al. (2010), the authors used CLSM and optical coherence tomography (OCT) to investigate heterotrophic biofilms. They showed that structural information about the biofilms gathered at the microscale (CLSM) did not accurately represent mesoscale measurements made with OCT.

As mentioned, the initial aim of the experiment was to see if particle size could be linked with particle location within the biofilm. In the end however, the limited

number of scans did not provide enough information for quantifying the position and size of settled wastewater cells within the biofilm.

Several studies have been performed to investigate the deposition of different abiotic and biotic particles in biofilm systems (Battin et al., 2003; Langmark et al., 2005; Searcy et al., 2006; Wolyniak et al., 2009, 2010). Searcy et al. (2006) performed experiments in small flow cells to learn more about the capture and retention of *Cryptosporidium* oocysts (approximately 5 µm) by biofilms. The authors found that oocyst attachment was positively correlated with biofilm roughness. In their case, as the roughness coefficient increased from approximately 0.3 to 0.8, the fraction of oocysts retained by the biofilm also increased from roughly 4 to 30 %. In a similar set of experiments, Wolyniak et al. (2009) identified oocyst attachment rates of greater than 40 %. The biofilms in the present study were characterized by an average roughness coefficient of 1.0 ± 0.2. Thus, the fraction of wastewater cell accumulation identified in this work (3.6 % after 24 h) is in comparison to the other two studies, considerably lower. Here, however, it should be considered that the other two studies worked with small flow cells having water depths less than 5 mm. Whether such small-scale systems are capable of producing representable deposition data which can be translated to large-scale systems is questionable. Moreover, the authors calculated these attachment fractions by simply taking the difference between the number of oocysts in the influent and effluent. In doing so, they neglected possible attachment to the walls of the flow cell and tubing which could have led to an overestimation of what was actually retained in the biofilm.

Battin et al. (2003) performed a set of experiments in large-scale flumes to investigate whether biofilm growth can change the hydrodynamic transient storage and organic matter processing at the interface between the streambed and overlying water. As part of this study, the authors explored the relationship between biofilm growth and the deposition velocity of suspended organic particles 53 - 106 µm in size. Interestingly, they found that the hydraulic exchange velocity was 5 - 8 times lower than the particle deposition velocities, indicating that advective transport could not have been the predominant factor in deposition. They thus concluded that "biofilm stickiness" must therefore play a decisive role in particle deposition in streams.

3.4 Summary

In this chapter, results are presented which report about the removal of FIB and wastewater cells from the water column in lab-scale Flume A. Attempts were made to more closely understand *E. coli* and enterococci deposition out of the water column and subsequent attachment to the benthic biofilm. Moreover, through use of fluorescently-labeled wastewater together with epifluorescence and confocal laser scanning microscopy, the overall rate of deposition and attachment of wastewater cells could be identified.

From this group of experiments, the following is concluded:

- FIB dark removal rates from the water column during the first 24 h, as determined with a culture-based technique, were approximately the same ($k_{ec} = 0.05\,\mathrm{h}^{-1}$; $k_{ent} = 0.06\,\mathrm{h}^{-1}$). However, from these results it was not possible to determine the individual contributions of natural inactivation and deposition on the overall removal.

- It was determined that within the first 24 h, 30 % of the FIB had been removed from the water column. Through a set of further experiments, it was seen that of this 30 %, 83 % of the removal occurred due to natural inactivation and 17 % from deposition onto the benthic biofilm.

- An FIB-specific deposition velocity of $5 \times 10^{-6}\,\mathrm{m\,s}^{-1}$ was determined which was notably slower than what Battin et al. (2003) and Jamieson et al. (2005a) determined for particle sizes ranging from 45 - 106 µm. However as the majority of FIB present in the wastewater used were associated with particle sizes $\leq 12\,\mu\mathrm{m}$, it is plausible that deposition velocities of FIB would be smaller.

- With the help of epifluorescence microscopy, the removal of fluorescently-labeled wastewater particles out of the water column of the lab-scale flume was monitored. Here it was seen that the deposition rate of CFDA-stained wastewater cells ($k_{D,CFDA}$) was $0.05\,\mathrm{h}^{-1}$.

- Through use of CLSM, the attachment of fluorescently-labeled wastewater particles to the benthic biofilm was examined. Merely 4 % of what was removed from the water column was actually detected in the benthic biofilm. Attempts to determine the recovery rates of wastewater particles in the biofilm indicated that only 12 % of what was theoretically expected, was actually found in the biofilm. This suggests that microscale information gathered from CLSM image stacks cannot necessarily be extrapolated to interpret processes which occur at the mesoscale.

Chapter 4

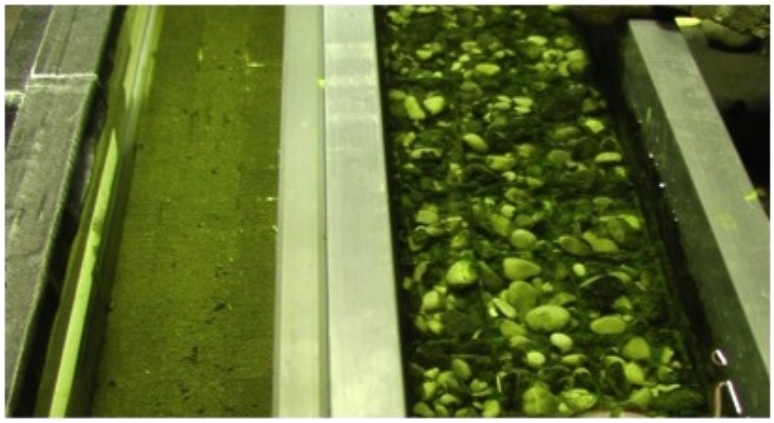
Persistence of Fecal Indicator Bacteria in Sediment of an Oligotrophic River: Comparing Behavior in Lab-Scale and Large-Scale Systems

In this chapter, investigations were performed in lab-scale Flume A and large-scale Flume B to determine the fate of *E. coli* and enterococci in the bed sediments of an oligotrophic river. Armed with this knowledge, in-stream water quality models can be adjusted to properly account for this compartment as a possible source or sink of FIB. By using two systems varying significantly in scale, the ability of relating lab-scale results to large-scale systems was additionally explored.

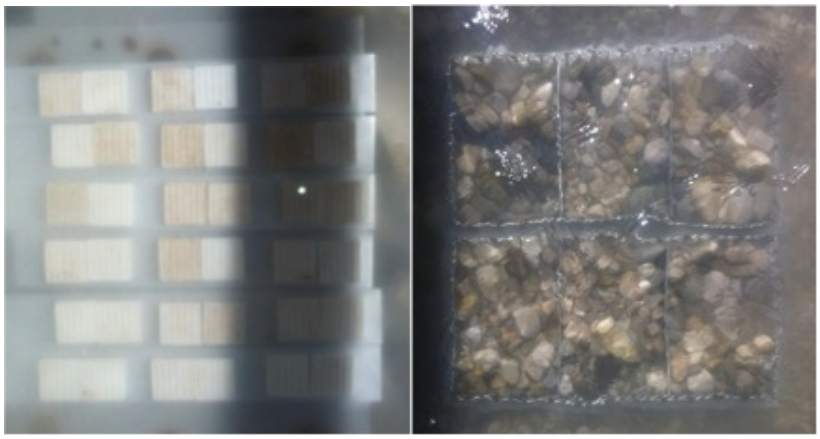
4.1 Development of benthic substrata in Flumes A and B

In FIGURE 4.1, images of the ceramic tiles (left) and substratum cages (right) in the lab and large-scale flumes are shown after approximately four weeks of cultivation. It is clearly evident that the benthic zones developed differently. The substratum cages and tiles in Flume A were predominately covered with algal biomass due to the shallow water column and UV lamps, which led to an average $oDM_{sediment}$ above 95 %. In Flume B however, a significant amount of inorganic sediment was present which resulted in an average $oDM_{sediment}$ of less than 5 %.

The biofilm that formed on the tiles in Flume A was markedly thicker than that which developed in Flume B. The mean biofilm thicknesses as determined by confocal laser scanning microscopy were approximately 200 and 40 µm in Flumes A and B, respectively. Although the water and substratum were nearly identical in both systems, the fact that the biofilms differed from each other is a result of the difficulty in mimicking natural conditions in a lab setting.



(a) lab-scale Flume A; Experiments $AIV_{a,b}$ and AV



(b) large-scale Flume B; Experiment BIII

FIGURE 4.1: Images of the ceramic tiles (left) and substratum cages (right) in the lab (a) and large-scale (b) flume systems.

4.2 Physico-chemical characteristics during flume experiments

The averaged physical and chemical parameters monitored throughout the flume experiments are presented in TABLE 4.1. It can be seen that the pH was very similar in both systems and that the water temperatures were significantly colder than what enteric microorganisms are accustomed to (37 °C).

On average, the COD_{water} and TSS_{water} were comparable between the two systems, however in large-scale Flume B there were spikes in both parameters at the end of day 1 due to heavy rain (TSS_{water} 113 mg l^{-1}; COD_{water} 17 mg l^{-1}). This rise in TSS_{water} led to a slight increase in the overall $DM_{sediment}$ as seen in FIGURE 4.2. The TSS_{water} concentrations are not depicted in FIGURE 4.2a as samples were only taken at the start and end of the experiments. The concentrations measured were always below 10 mg l^{-1} (see TABLE 4.1).

TABLE 4.1: Average physico-chemical parameters in Flumes A and B.

parameter	unit	lab-scale Flume A	large-scale Flume B
T_{water}	C	12	7.8
COD_{water}	$mg\,l^{-1}$	<10	<10*
pH	-	8.2	8.4
TSS_{water}	$mg\,l^{-1}$	<10	<10*
$DM_{sediment,tile}$	$g_{DM}\,m^{-2}$	43	112
$DM_{sediment,cage}$	$g_{DM}\,m^{-2}$	87	2542
$oDM_{sediment}$	%	>95	<5

* excluding t = 1 - 2.5 d

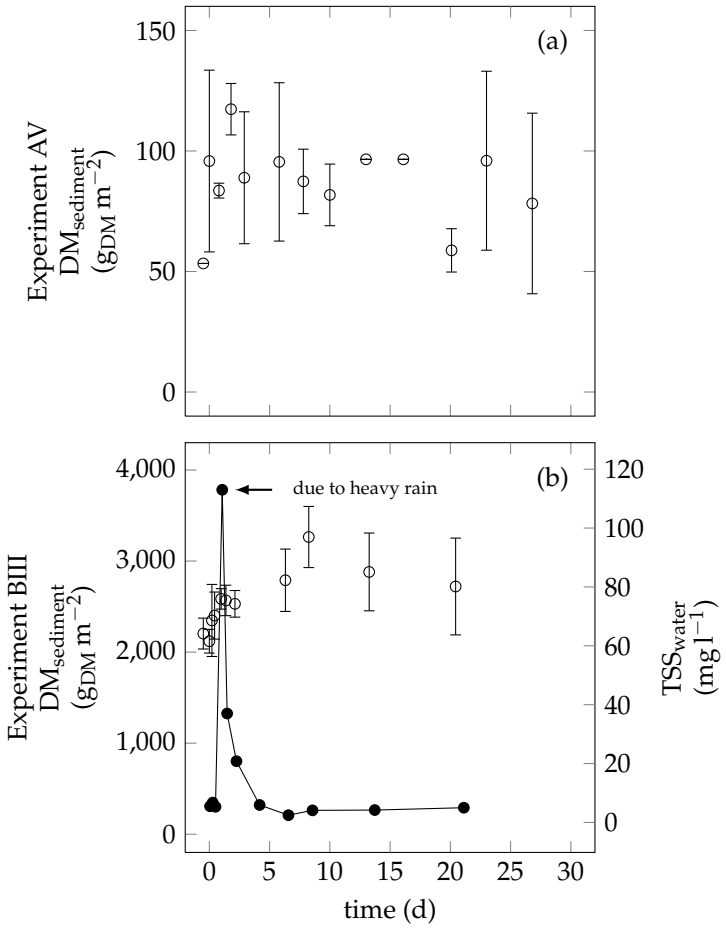


FIGURE 4.2: (a) Average $DM_{sediment}$ measured in substratum cages during lab-scale Experiment AV. (b) Average $DM_{sediment}$ (○) in substratum cages and TSS concentrations (●) in the water column during the large-scale Experiment BIII.

4.3 Fate of FIB in the benthic zone

The results of the large and small-scale flume experiments are depicted in FIGURE 4.3 and are discussed in more detail in the following sections.

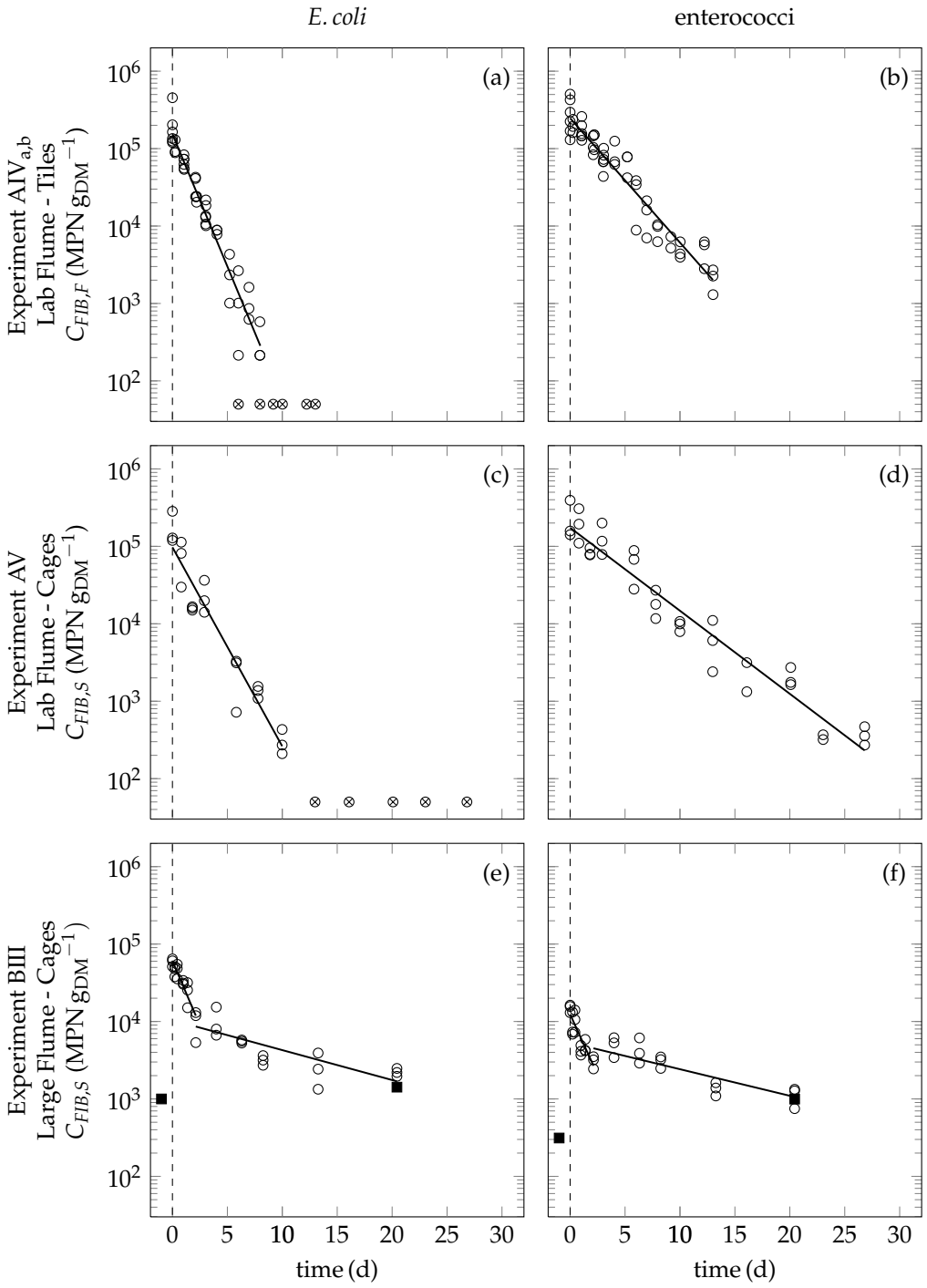


FIGURE 4.3: Measured *E. coli* and enterococci concentrations in the flume bed throughout the persistence experiments in: Flume A with tiles (a, b), Flume A with substratum cages (c, d), and Flume B with substratum cages (e, f). Values below the method detection limit are depicted by ⊗. ■ indicates mean background (before wastewater addition) and end concentrations. First-order trend lines have been inserted throughout; dashed lines indicate start of experiment.

4.3.1 Persistence of FIB in biofilm formed on ceramic tiles in Flumes A and B (Experiments AIV$_{a,b}$ and BIII)

In FIGURE 4.3a and b, the fate of *E. coli* and enterococci in the benthic biofilm formed on the tiles of lab-scale Flume A is presented. The background FIB concentrations in the biofilm (data not shown) were below the method detection limit. After inoculating the system for 24 h with wastewater (*E. coli*: $2.2 - 4.7 \times 10^4$ MPN ml^{-1}; enterococci: $1.0 - 1.1 \times 10^4$ MPN ml^{-1}), initial concentrations in the biofilm (t = 0 d) ranged between 1.3×10^5 and 5.1×10^5 MPN $g_{DM}{}^{-1}$. From FIGURE 4.3a and b, it is evident that both FIB types displayed first-order decay rates in the biofilm and that any growth which may have occurred, did not lead to an accumulation. Dark removal rate constants for *E. coli* and enterococci in the biofilm were 0.8 and 0.4 d^{-1}, respectively.

To keep FIGURE 4.3 to a manageable size, results for the ceramic tiles in large-scale Flume B have been left out, but are described in the following. Average background *E. coli* and enterococci concentrations in the biofilm after the four-week cultivation period measured 6.6×10^3 and 8.0×10^2 MPN $g_{DM}{}^{-1}$, respectively. Following overnight inoculation of the system with wastewater, 2-log spikes in the benthic concentrations were seen for both FIB types. Throughout the first 2 d of the experiment, first-order decay trends were observed and similar rate coefficients were determined for both FIB types: $k_{ec} = 1.2$ d^{-1} ($R^2 = 0.87$) and $k_{ent} = 1.5$ d^{-1} ($R^2 = 0.93$).

4.3.2 Persistence of FIB in substratum cages in Flumes A and B (Experiments AV and BIII)

In FIGURE 4.3c and d, the persistence of *E. coli* and enterococci in the substratum cages of lab-scale Flume A is presented. The background conditions within the system (not shown) were below the method detection limit. Upon addition of wastewater to the system, concentrations in the substratum cages peaked at approximately 10^5 MPN $g_{DM}{}^{-1}$. Thereafter, a first-order removal rate was observed where the removal rate coefficients for *E. coli* and enterococci were 0.6 and 0.3 d^{-1}, respectively. As can be seen in FIGURE 4.3d, even after 27 d enterococci were still present in the substratum cages, nearly twice as long as *E. coli*.

FIB persistence in the substratum cages of large-scale Flume B is depicted in FIGURE 4.3e and f. In contrast to the lab-scale experiments, there were detectable concentrations of *E. coli* and enterococci in the substratum cages before the start of the experiment ($t = -0.5$ d). The average background *E. coli* and enterococci concentrations were 1.0×10^3 and 3.1×10^2 MPN $g_{DM}{}^{-1}$, respectively, and are depicted by black squares. Due to the inoculation overnight with wastewater, FIB concentrations in the biofilm/sediment at the start of the experiment were nearly two logs higher. Throughout the first 2.5 d of the experiment, similar first-order decay coefficients were observed

for both FIB ($k_{ec} = 0.8\,\mathrm{d}^{-1}$; $k_{ent} = 0.7\,\mathrm{d}^{-1}$). However, from 2.5 d onwards the decay of *E. coli* and enterococci slowed considerably ($k_{ec} = 0.09\,\mathrm{d}^{-1}$; $k_{ent} = 0.08\,\mathrm{d}^{-1}$).

In addition to the standard MPN technique, enterococci concentrations in the substratum cages of both the lab and large-scale flumes were also analyzed with qPCR. The results are presented in FIGURE 4.4, whereby the background concentrations in each system are shown at ($t = -0.5$ d). Following the addition of wastewater, there was roughly a 2-log increase in the benthic concentrations. It is more important to consider the trends of the two experiments rather than comparing concentrations between them.

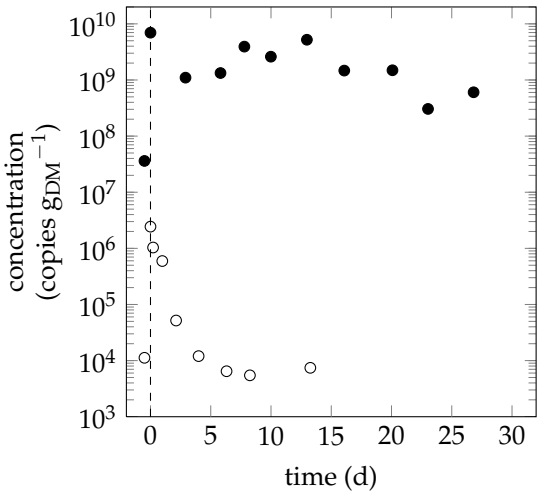


FIGURE 4.4: Enterococci concentrations measured in the substratum cages of the lab-scale (●; Experiment AV) and large-scale (○; Experiment BIII) flumes as determined by qPCR. The dotted line indicates the start of the experiment.

While the values in lab-scale Flume A remained fairly constant over the 27 d experiment, we see that in large-scale Flume B there was a rapid decrease in concentration during the first 4 d, after which the concentration reached the background level.

4.4 Influence of benthic substrata on FIB persistence in lab and large-scale flumes

In an attempt to understand the potential for different benthic substrata to act as a reservoir of pathogens, the persistence of FIB in both 3-cm-deep substratum cages and a thin biofilm was investigated. It is well known that for a variety of reasons including a higher availability of soluble organic matter and nutrients, as well as protection from protozoa, predators, and UV light rays, bed sediments can represent a hospitable environment in which autochthonous microorganisms can survive longer than in the water column (Gerba and McLeod, 1976; Davies et al., 1995; Davies and Bavor, 2000; Jamieson et al., 2005a,b).

As the substratum cages used in this study were considerably deeper than the thin biofilm (µm) coating the ceramic tiles, it would seem apparent that the 3-cm-deep cages would offer more protection and thus, promote longer FIB survival. With the results of the four flume experiments presented in SECTIONS 4.3.1 and 4.3.2 and summarized in TABLE 4.2, it becomes interesting to compare the role that the substratum type had on the FIB removal rate coefficient. In lab-scale Flume A, no perceivable difference was noted between the persistence of *E. coli* and enterococci on the tiles and in the substratum cages. However, the anticipated trend of longer persistence in the sediment cages was seen in large-scale Flume B. There, *E. coli* was removed 1.5 times faster from the ceramic tiles than from the substratum cages (1.2 vs. $0.8\,d^{-1}$) and enterococci more than twice as fast (1.5 vs. $0.7\,d^{-1}$).

TABLE 4.2: Summary of *E. coli* (ec) and enterococci (ent) removal rate coefficients (k) in benthic sediment/biofilm.

experiment	flume	scale	substratum	k_{ec} (R^2) (d^{-1})	k_{ent} (R^2) (d^{-1})
$AIV_{a,b}$	A	lab	tile	0.8 (0.98)	0.4 (0.96)
AV	A	lab	cage	0.6 (0.92)	0.3 (0.94)
BIII*	B	large	tile	1.2 (0.87)	1.5 (0.93)
BIII*	B	large	cage	0.8 (0.92)	0.7 (0.88)
BIII**	B	large	cage	0.09 (0.80)	0.08 (0.78)

*t = 0 - 2.5 d, **t = 2.5 d onwards

The question which arises from these results is why in the lab setting is the expected trend of longer FIB persistence in substratum cages not observed. Why is FIB survival on tiles the same as in substratum cages at the lab-scale, but in the large-scale flume the awaited trend occurs? One possible explanation may be related to the water used in both systems. In the lab-scale flume, river water was pre-filtered (100 µm sieve) to remove large particles and larvae. Due to this filtering step, grazing/predation most likely did not factor into FIB removal from the benthic zone in lab-scale Flume A. Thus in Flume A, whether the FIB were situated in deeper layers of the substratum cages or located in the thin biofilm on the ceramic tiles, was irrelevant with respect to protection from grazing. This was most certainly not the case in the large-scale flume as fresh river water was used and a variety of larvae including those of caddisflies, black flies, stoneflies, and mayflies were spotted.

An additional explanation for the more rapid removal from the biofilm-covered tiles in Flume B may be connected to the considerably different bed shear stresses in the two flumes. In large-scale Flume B, the bed shear stress was roughly $9\,N\,m^{-2}$, which very closely approximates values measured in the Isar River under normal conditions. In lab-scale Flume A however, only $0.3\,N\,m^{-2}$ could be achieved. In deeper regions of the substratum cages, the bed shear stress would have been negligible. As a result, FIB

in these regions would have been more protected from such adverse hydrodynamic conditions. In the case of Flume B where the bed shear stress was comparatively high, it is logical that FIB would have had greater survival chances if located deeper within the substratum cage rather than on the surface of the biofilm-coated ceramic tile. This finding illustrates that for systems characterized by the presence of grazers/predators and high bed shear stresses, it is necessary to consider FIB survival within a specific sediment thickness and not simply on the upper surface of the benthic substratum.

4.5 Mechanisms of FIB removal in lab-scale versus large-scale flumes

Several studies were identified in literature which used natural sediments to investigate the persistence of FIB, however the experiments were typically conducted in batch setups and often without flow. In order to get accurate information about FIB removal from bed sediments, it was deemed necessary to conduct experiments in both large and lab-scale flume systems.

4.5.1 Removal behavior until day 2.5

In TABLE 4.2, it can be seen that k_{ec} determined in the lab for both substrata (0.8 and 0.6 d^{-1}) accurately approximated the removal rate coefficient in the substratum cages of large-scale Flume B during the first 2.5 days. These values for *E. coli* all fall within the range measured in soil or bed sediments of 0.02 - 1.1 d^{-1} as found in literature (Reddy et al., 1981; Craig et al., 2002; Jamieson et al., 2005b; Haller et al., 2009; Garzio-Hadzick et al., 2010). For the case of enterococci, the results from lab-scale Flume A differ significantly from those in large-scale Flume B. Specifically, k_{ent} in the substratum cages of Flume B was 2.3 times faster than in Flume A. This discrepancy was even more pronounced for the biofilm-covered ceramic tiles. Here, removal rate coefficients were 3.75 times faster in the natural system compared to the lab-scale flume. Both k_{ent} values of 0.7 and 1.5 d^{-1} (see TABLE 4.2) are appreciably out of the range of rates (0.04 - 0.41 d^{-1}) as determined by other groups (Reddy et al., 1981; Craig et al., 2002; Jamieson et al., 2005b; Haller et al., 2009; Garzio-Hadzick et al., 2010).

In order to shed more light on this discrepancy, enterococci concentrations in the substratum cages of both lab and large-scale flumes were determined with qPCR. As seen in FIGURE 4.4, while enterococci were rapidly removed from the large-scale flume, their concentrations (copies $g_{DM}{}^{-1}$) remained constant for 27 d in the lab-scale flume. This indicates that the removal mechanisms for FIB are different in the lab and large-scale flumes. In the lab-scale flume where grazers/predators were presumably not present, we see that FIB removal (as measured with a culture-based technique) oc-

curred due to inactivation, most likely because of poor nutrient levels and low water temperature; their DNA though was still present in the system. Thus, the DNA appears to be very stable, even when the cells are no longer active enough to be cultured. However as seen in FIGURE 4.4, FIB were physically eliminated from the large-scale flume system. Therefore, removal rates in Flume B were most likely due to the combined effect of consumptive and non-consumptive losses from grazing/predation as well as washout from the system (*e.g.* erosion of bed sediments due to high shear stress).

This result clarifies why the enterococci removal rate coefficient matches that of *E. coli* in the large-scale flume, but not at the lab-scale (see TABLE 4.2). It appears that in a system without grazers/predators and having an unrealistically low bed shear stress, enterococci survival surpasses that of *E. coli* based solely on the specific traits of the microorganism (gram-positive, more robust cell wall). This prolonged survival of enterococci in comparison to *E. coli* has been repeatedly observed by other research groups (Reddy et al., 1981; Haller et al., 2009). However, when benthic invertebrates are present in an ecosystem having a high bed shear stress, consumptive and non-consumptive losses due to grazing/predation as well as washout become the dominant factors in removal, irrespective of FIB type. Thus, FIB removal rates determined in systems operated in the absence of such invertebrates and at low bed shear stresses will result in overly conservative approximates of FIB concentrations.

4.5.2 Removal behavior after day 2.5

From the results presented in FIGURE 4.3e and f and TABLE 4.2, it can be seen that after day 2.5, FIB removal slowed considerably until the end of the experiment. Contrary to this, removal in the lab-scale flume proceeded at a constant rate until either the experiment was ended or FIB were no longer detected with the MPN technique (see FIGURE 4.3a - d). One possible explanation for the trend in large-scale Flume B is that the FIB of wastewater origin were not uniformly distributed within the substratum cages following inoculation, but were perhaps more concentrated in the upper layers. Once this upper layer of settled FIB was removed due to grazing and resuspension/washout (first 2.5 d), and assuming that the wastewater FIB were more active than background FIB in the flume, the slowed k-values of 0.09 and 0.08 d^{-1} may have been the result of diminishing culturability of the wastewater FIB located deeper within the substratum cages over time.

One additional explanation for the stagnation is that FIB were transported into the system because large-scale Flume B was operated in flow-through mode. The spike in TSS at day 1 (see FIGURE 4.2) and subsequent reduction until day 4, may have possibly led to the deposition of TSS-associated FIB onto the bed sediments during this period. Indeed, there was an increase in the measured $DM_{sediment}$ after day 4 (see FIGURE 4.2)

which indicates that some degree of deposition of TSS from the water column to the flume bed occurred. This increase in $DM_{sediment}$ was however not high enough to have had a dilution effect on the comparatively high FIB levels and thus, did not work to slow the removal rates down.

Nonetheless, despite the slowed removal after day 2.5, the removal rate coefficients measured from 0 - 2.5 d appear to be representative values for an oligotrophic stream. Obviously one drawback of such a large-scale, open system is the limited control over fluctuations in influent water quality. However, the benefits (*e.g.* representative hydraulic conditions and benthic development) seem to outweigh this disadvantage.

4.6 Summary

In the present work, lab and large-scale flume experiments were performed to gain insight into the survival of FIB in the benthic zone of an oligotrophic river. The impact of different substrata as well as bed shear stress on FIB persistence were explored. Finally, the ability of lab-scale systems to emulate natural systems was investigated. From this study, the following conclusions have been drawn:

- Large-scale flume systems represent an invaluable tool for investigating the persistence of FIB in the bed sediments of an oligotrophic alpine river as they provide more realistic conditions than lab flumes.
- Bed shear stress is an important factor to consider when constructing flumes as it appears to play a role in FIB persistence in upper layers of riverbed sediments. Additionally, it is imperative to examine FIB survival throughout the top 3 cm of sediments and not simply on the upper layer.
- It was seen that grazing and/or washout of FIB were the predominant removal mechanisms in the large-scale system operated with a realistic bed shear stress. Contrary to this, in the lab-scale flume having an unrealistically low bed shear stress and no grazers, enterococci persisted twice as long as *E. coli*. Their removal was a result of inactivation due to the low temperature and nutrient concentrations.

Chapter 5

Influence of Resuspension on the Fate of Fecal Indicator Bacteria in Large-Scale Flumes Mimicking an Oligotrophic River

5.1 Standard removal experiments with a constant discharge (Experiments $BI_{a,b}$)

Two experiments were conducted large-scale Flume B to determine standard removal patterns of the fecal indicator organisms *E. coli* and enterococci from the water column after a simulated CSO. The discharge was held constant throughout the experiments at $0.2\,m^3s^{-1}$, which corresponded to a bed shear stress typical for the Isar River of approximately $9\,N\,m^{-2}$.

5.1.1 Physico-chemical parameters

In TABLE 5.1 and TABLE 5.2, the characteristics within the flume and for the raw wastewater used are presented, respectively. The UV parameter indicates the average sunlight intensity at the top of the water column. It can be seen that the experiments were conducted under very similar conditions. Sunlight intensity and water temperature were the two parameters that varied slightly between runs; oxygen saturation of the water was always near 100 % and pH approximately 8.3.

The background COD concentrations in the Obernach River before the CSO for the two experiments were below $10\,mg\,l^{-1}$. After addition of raw wastewater, the COD jumped to roughly 16 $mg\,l^{-1}$ and decreased exponentially. Within approximately 5 h the COD had dropped below $10\,mg\,l^{-1}$. TOC levels in the water column before Experiments $BI_{a,b}$ were below $2\,mg\,l^{-1}$. Following the addition of raw wastewater, the TOC

TABLE 5.1: Flume/experiment characteristics during standard removal Experiments $BI_{a,b}$.

Parameter	Unit	Experiment BI_a	Experiment BI_b
Experiment Start	DD.MM.YY	18.10.10	09.05.11
Experiment End	DD.MM.YY	21.10.10	12.05.11
T_{water}	°C	10.0	15.7
$COD_{background}$	$mg\,l^{-1}$	9	7
$TOC_{background}$	$mg\,l^{-1}$	1.7	1.4
$^*UV_{290-390\,nm}$	$W\,m^{-2}$	2.9	6.5
Q	$m^3\,s^{-1}$	0.2	0.2
τ_b	$N\,m^{-2}$	9	9
water depth	m	0.5	0.5
mean velocity	$m\,s^{-1}$	0.9	0.8
$TSS_{background}$	$mg\,l^{-1}$	8	4

*mean daytime value

TABLE 5.2: Characteristics of wastewater used in Experiments $BI_{a,b}$.

Parameter	Unit	Experiment BI_a	Experiment BI_b
COD	$mg\,l^{-1}$	183	169
TOC	$mg\,l^{-1}$	58	21
$C_{E.\,coli}$	$MPN\,ml^{-1}$	2.5×10^4	3.2×10^4
$C_{enterococci}$	$MPN\,ml^{-1}$	4.4×10^3	5.3×10^3
TSS	$mg\,l^{-1}$	55	47

concentrations increased and subsequently decreased in a similar manner as COD.

Initial TSS concentrations of 4 - 8 $mg\,l^{-1}$ were measured in the water, which increased to 20 - 25 $mg\,l^{-1}$ after switching into recirculation mode and the introduction of raw wastewater. The TSS decreased exponentially until approximately $t = 20$ h at which point it had returned to the background concentration.

5.1.2 *E. coli* and enterococci removal from the water column

E. coli and enterococci concentrations during the first 50 h of the two standard removal experiments are depicted in FIGURE 5.1. In addition to the average water temperature and daytime UV intensity throughout the first 30 h of each experiment, first-order removal rate coefficients together with their respective coefficients of determination have been included. The background FIB concentrations in the water column (not shown) were at or below the method detection limit of 3.8×10^{-1} $MPN\,ml^{-1}$. The high initial concentrations seen in FIGURE 5.1 at $t = 0$ h resulted from the point loading of raw wastewater.

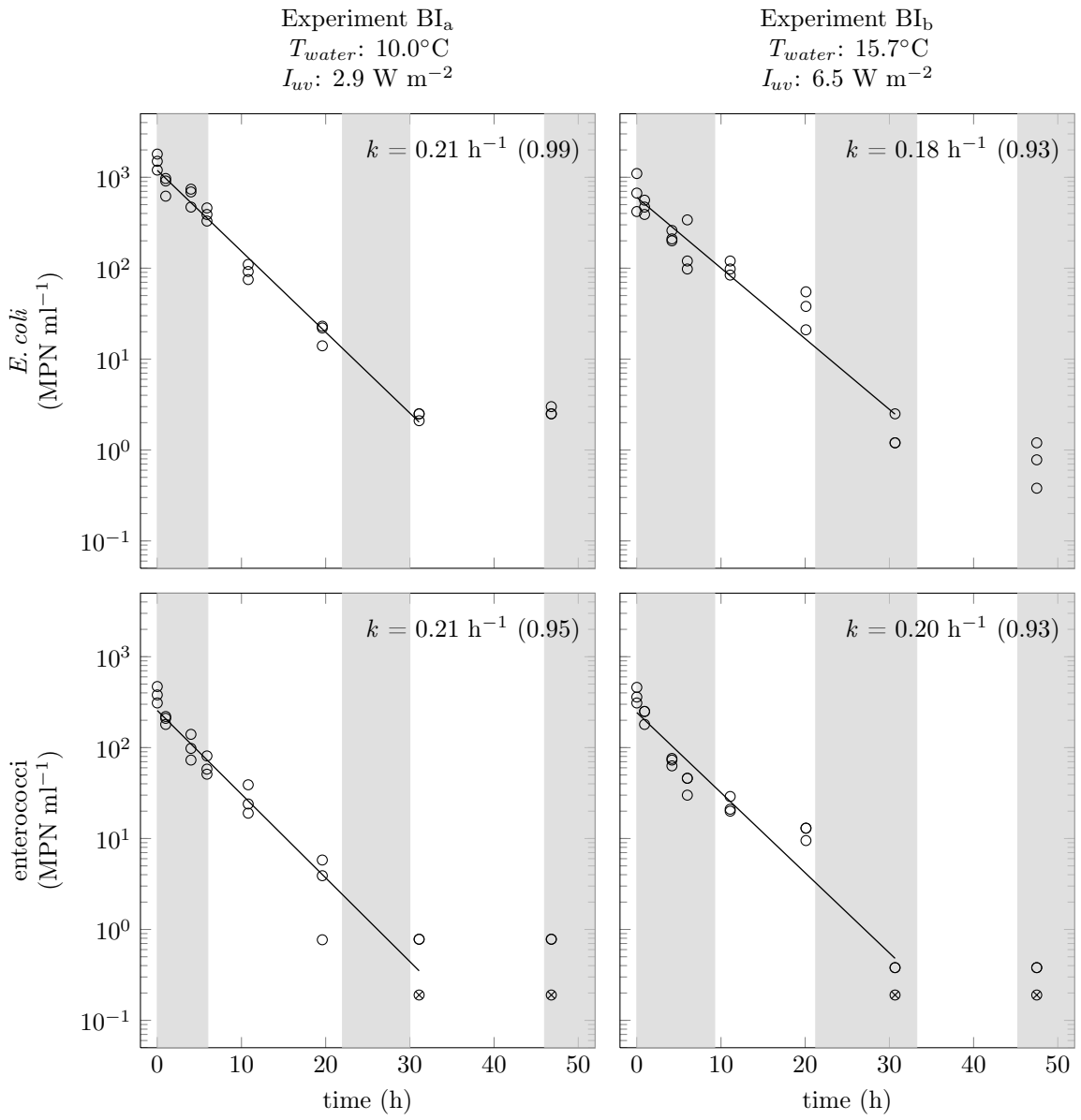


FIGURE 5.1: Fate of *E. coli* and enterococci in the water column during standard removal Experiments BI$_{a,b}$. Shaded regions indicate periods when the flume was exposed to sunlight. First-order trend lines together with the corresponding removal rate coefficients (k) have been included; coefficients of determination are provided in parentheses. ⊗ indicate that concentrations were below the detection limit.

As seen in FIGURE 5.1, *E. coli* and enterococci removal occurred at a constant rate during the first 30 h following the simulated CSO. First-order removal rate coefficients for *E. coli* and enterococci during this period ranged between 0.18 and 0.21 h^{-1}, whereby no appreciable difference between the enteric microorganisms was seen. Except for *E. coli* in Experiment BI$_a$, after the first 30 h the FIB concentrations were either approaching or had reached the method detection level. In Experiment BI$_a$, the *E. coli* concentration leveled off at 2 MPN ml^{-1} and remained so until the end of the experiment after 72 h. As the concentrations of *E. coli* in the water column were higher than those of enterococci following the simulated CSO, it took longer for *E. coli* to reach the

detection limit.

Removal rate coefficients of FIB, often referred to as die-off or inactivation coefficients, have been widely investigated for their dependence on certain biotic and abiotic conditions (*e.g.* temperature, pH, salinity, predation, solar irradiance). Published values were found to vary between 0.008 and $0.95\,h^{-1}$ depending on which condition was being explored (Sinton et al., 2002; Menon et al., 2003; Chigbu et al., 2005; Servais et al., 2007; Schultz-Fademrecht et al., 2008; Mitch et al., 2010). For example, Schultz-Fademrecht et al. (2008) investigated the impact of sunlight on inactivation of fecal coliforms and enterococci in river water and reported k values of 0.1 - $0.95\,h^{-1}$. Specifically, at a UV intensity of $8.0\,W\,m^{-2}$ the study determined the removal rate of enterococci and fecal coliforms to be 0.4 and $0.5\,h^{-1}$, respectively. When the UV intensity was lowered to near-dark conditions of $0.8\,W\,m^{-2}$, k dropped to $0.1\,h^{-1}$ for both enterococci and fecal coliforms. The fact that in the present study no appreciable difference was found between the removal rates for *E. coli* and enterococci reflects this finding of Schultz-Fademrecht et al. (2008) at near-dark conditions. Moreover, the average removal rate coefficient of *E. coli* and enterococci ($0.2\,h^{-1}$) falls within the range of dark and light coefficients determined by Schultz-Fademrecht et al. (2008). However, in this study no significant difference in removal during daytime and nighttime was seen. This is most likely due to the fact that although the sun was out for at least 7 h a day, due to the relatively narrow channel width (50 cm) compared to wall height (60 cm), the time that the sun shined directly on the flume was minimal. Thus, although UV inactivation is considered to be one of the main FIB inactivation mechanisms, in these flume systems it played a lesser role in the water column removal rates. This was advantageous as it was then feasible to more closely investigate the different FIB removal mechanisms such as inactivation due to hostile conditions the water column provides (low temperature and nutrients) as well as deposition/sedimentation out of the water column.

It becomes interesting to compare these results with those obtained from experiments performed in the lab-scale flume (SECTION 3.2.1). The removal rate coefficients determined in the lab set-up ranged between 0.05 and $0.06\,h^{-1}$, considerably slower than the approximate value of $0.2\,h^{-1}$ as seen in the outdoor flume. Moreover, the rate of $0.2\,h^{-1}$ is significantly faster than rates found in literature. For example, Servais et al. (2007) conducted experiments with *E. coli* in a batch system using water collected from the Seine River. Following inoculation of their system with a strain of tritiated-thymidine-labeled *E. coli*, samples were periodically taken and mortality was determined based on the disappearance of radioactivity from the DNA; the authors determined a mortality rate coefficient of $0.03\,h^{-1}$. Obviously as a culture-based method was used in the current work, rates are expected to be higher due to losses in culturability. Sinton et al. (2002) performed experiments to determine decay rates of wastewater FIB in fresh river water with a culture-based technique. The authors again

worked with batch systems, although larger in size (300 l), which were however mixed with a submersible pump. Dark inactivation rate coefficients of $0.02\,h^{-1}$ and $0.01\,h^{-1}$ were determined for *E. coli* and enterococci, respectively, very similar to the findings of Servais et al. (2007) who did not use a culture-based technique.

Clearly it is difficult to compare *k*-rates between different studies due to the broad range of environmental conditions associated with each set-up. Nevertheless, we are of the opinion that importance should be placed on performing such investigations in systems which accurately represent natural streams. As shear stress can impact FIB persistence in flowing systems (CHAPTER 3; Sichel et al. (2007)), such large-scale flumes which mimic actual stream hydraulics may provide more realistic information not obtainable in batch experiments.

5.1.3 Deposition of *E. coli* and enterococci onto the riverbed

In addition to determining FIB concentrations in the water column, biofilm-covered rock samples were also analyzed to ascertain if deposition played a role in the removal rate coefficients determined in section SECTION 5.1.2. The results are depicted in FIGURE 5.2 and are related to the surface areas of the stones. Background concentrations for *E. coli* ranged between 5×10^{-1} and $1.24 \times 10^{2}\,MPN\,cm^{-2}$ and for enterococci from 3×10^{-1} to $2 \times 10^{1}\,MPN\,cm^{-2}$. These concentration ranges initially found in the biofilm before CSO simulation are illustrated in FIGURE 5.2 by the shaded regions. This wide concentration range indicates that FIB distribution over the riverbed is not uniform. The Obernach River is an alpine river which at certain reaches travels through agricultural fields/cow pastures. There are however no wastewater treatment plants that discharge into the river. The residual FIB concentrations found initially in the biofilm before the CSO simulation most likely can be attributed to agricultural runoff from these regions. As the flumes were run in flow-through mode for a minimum of three weeks prior to an experiment, it is assumed that the FIB entered the system during this cultivation phase.

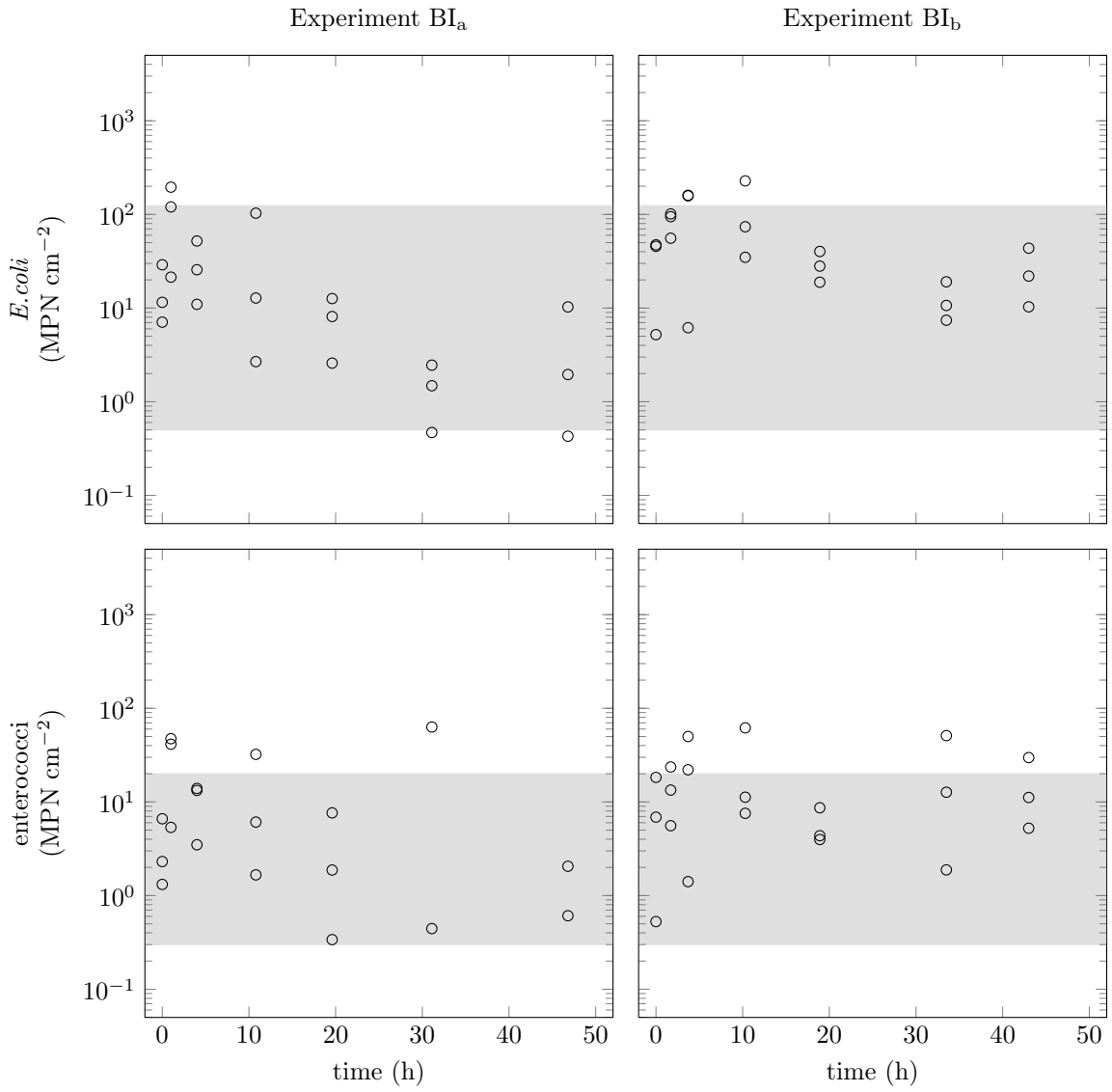


FIGURE 5.2: *E. coli* and enterococci concentrations in the benthic biofilm during standard removal Experiments BI$_{a,b}$. The shaded regions indicate the range of background concentrations determined in the biofilm before the experiment was started.

Throughout both experiments, the majority of the measured FIB concentrations in the biofilm fall within the range of background concentrations. In a lab-scale flume study mimicking an oligotrophic river, Schultz-Fademrecht et al. (2008) investigated the deposition of FIB onto benthic biofilms following CSOs. The authors determined that after 24 h, approximately 10 % of the FIB from the raw wastewater were found in the biofilm and were still culturable. In the present study, even if we assume that only 10 % of the initial FIB load from the 1 m^3 of raw wastewater (see TABLE 5.2) added to the system were deposited homogeneously onto the flume bed and still culturable, *E. coli* and enterococci concentrations in the biofilm should have at times at least measured 1×10^3 and 5×10^2 MPN cm^{-2}, respectively. There were a few instances where enterococci levels were greater than background levels which suggests a small degree of deposition. However, as *E. coli* concentrations never exceeded 3×10^2 MPN cm^{-2}

and enterococci 9×10^1 MPN cm^{-2}, it appears as if deposition of culturable FIB onto the flume bed played only a minor role in removal from the water column. Moreover, any deposition which did occur did not lead to a net accumulation of culturable FIB in the biofilm. As only biofilm-covered stones were analyzed, there is the possibility that more FIB were deposited onto the flume bed, but were rather associated with finer sediments below the gravelly bed. This trend has been shown before by Muirhead et al. (2004).

As enteric microorganisms are accustomed to the warm, nutrient-rich conditions of the human gut, Obernach River water (mean water temperatures during first two experiments of 10.0 and 15.7 °C, low nutrients) represents a hostile environment not necessarily conducive to their survival. Therefore, although a differentiation between removal from the water column by deposition, predation, loss of culturability due to unfavorable conditions, and UV inactivation cannot be made, from the results presented in FIGURE 5.1 and FIGURE 5.2 it is assumed that a combination of loss of culturability due to the low temperature and nutrient levels as well as predation were the predominant removal mechanisms.

5.2 Experiments to investigate the impact of bed shear stress on FIB persistence (Experiments $BII_{a,b}$)

After the standard removal patterns of *E. coli* and enterococci in the water column were ascertained, two further experiments (Experiments $BII_{a,b}$) were conducted to investigate the effect of an increased bed shear stress on the fate of FIB. Due to limitations in the volume of water that could be recirculated, the flumes were initially operated at a shear stress of approximately 3 N m^{-2}. After a certain length of time following the simulated CSO (Experiment BII_a: 2.3 h; Experiment BII_b: 3.2 h), resuspension of the fine bed sediments was induced by suddenly increasing the discharge. This led to an increase in the bed shear stress to roughly 9 N m^{-2} ; it took approximately 20 min before the flow rate stabilized. As the role of deposition in removal of enteric microorganisms from the water column appeared minimal in Experiments $BI_{a,b}$, resuspension of significant amounts of culturable FIB from the riverbed was not expected. However, during heavy rain events the load of suspended solids is significantly higher than during dry-weather flow. Therefore, by inducing resuspension of bed sediments an attempt was made to mimic the TSS loading during an intense rain event.

5.2.1 Physico-chemical parameters

The characteristics of the flume and raw wastewater used for Experiments $BII_{a,b}$ are presented in TABLE 5.3 and TABLE 5.4, respectively. As in the standard experiments,

the pH and oxygen saturation values of the water remained constant throughout the duration of the experiment. Q_{before} and Q_{after} represent the discharges before and after the resuspension event; $\tau_{b,before}$ and $\tau_{b,after}$ indicate the bed shear stress prior to and after the increased discharge. The intensity of the sunlight was on average higher for these two experiments compared to the standard experiments as they were conducted during the spring and summer months. However, as UV inactivation was deemed to play a minor role in inactivation in the flume system, this was not perceived as influential.

TABLE 5.3: Flume/experiment characteristics during resuspension Experiments $BII_{a,b}$.

		Experiment	
Parameter	Unit	BII_a	BII_b
Experiment Start	DD.MM.YY	27.06.11	18.07.11
Experiment End	DD.MM.YY	30.06.11	21.07.11
T_{water}	°C	19.7	16.1
$COD_{background}$	$mg\,l^{-1}$	9	<5
$TOC_{background}$	$mg\,l^{-1}$	1.6	5.6
$^*UV_{290-390\,nm}$	$W\,m^{-2}$	17	8.9
$Q_{before\ resuspension}$	$m^3\,s^{-1}$	0.07	0.11
$Q_{after\ resuspension}$	$m^3\,s^{-1}$	0.15	0.21
$\tau_{b,before\ resuspension}$	$N\,m^{-2}$	3	3
$\tau_{b,after\ resuspension}$	$N\,m^{-2}$	9	9

*mean daytime value

TABLE 5.4: Characteristics of added wastewater during resuspension Experiments $BII_{a,b}$.

		Experiment	
Parameter	Unit	BII_a	BII_b
COD	$mg\,l^{-1}$	118	111
TOC	$mg\,l^{-1}$	24	19
$C_{E.\,coli}$	$MPN\,ml^{-1}$	4.0×10^4	2.5×10^4
$C_{enterococci}$	$MPN\,ml^{-1}$	2.9×10^3	4.0×10^3
TSS	$mg\,l^{-1}$	33	52

Similar to Experiments $BI_{a,b}$, COD concentrations in the flume water before the simulated CSO were below $10\,mg\,l^{-1}$. Following the addition of raw wastewater, concentrations increased to approximately $12\,mg\,l^{-1}$ and began to decrease with time (see FIGURE 5.3). This initial decrease in COD can be seen more clearly in Experiment BII_b as almost an extra hour was allowed before resuspension (2.3 h in Experiment BII_a vs. 3.2 h in Experiment BII_b).

As soon as the flow rate was increased to induce resuspension, the COD concentrations increased again to roughly $15\,mg\,l^{-1}$. Thereafter, the COD did not immediately

begin to decrease towards the background level of <10 mg l^{-1}, but rather fluctuated between 10 and 15 mg l^{-1}. Only after approximately 20 - 30 h did the COD begin to drop below 10 mg l^{-1}. The background TOC concentration in Experiment BII$_a$ was 1.6 mg l^{-1}; a slightly higher value of 5.6 mg l^{-1} was detected in Experiment BII$_b$. Following the addition of raw wastewater, the TOC levels increased and began to decrease until the flow velocity was increased. Again, as was seen for COD, the TOC concentrations increased following the resuspension event and remained elevated for 20 - 30 h. Thereafter, they began to decrease back to the start concentrations.

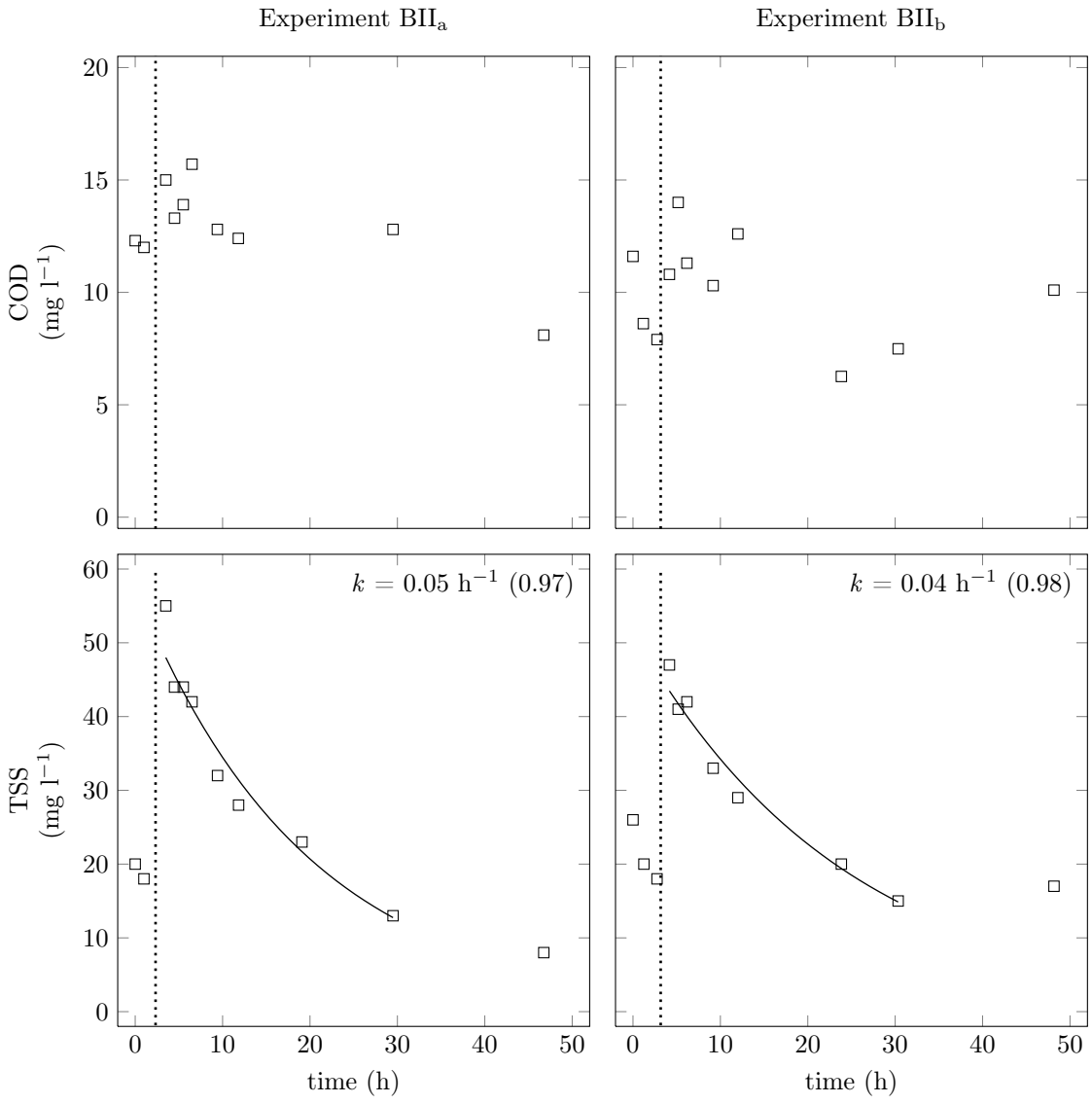


FIGURE 5.3: Effect of a sudden increase in bed shear stress on the COD and TSS concentrations in the water column during Experiments BII$_{a,b}$. Dotted lines indicate when the flow rate was increased. First-order trend lines are depicted for TSS with solid lines.

5.2.2 TSS characteristics

In FIGURE 5.3, the TSS concentrations for Experiments $BII_{a,b}$ are also depicted. The dotted lines represent the time at which the discharge was increased. The background TSS concentrations of the Obernach River (not shown) were similar to those in the standard removal experiments ($< 10\,mg\,l^{-1}$). The same effect was seen in Experiments $BII_{a,b}$ that when raw wastewater was added as a point load, the TSS values increased to more than $20\,mg\,l^{-1}$. The effect of resuspension is clearly illustrated in both plots by the distinct rise in TSS immediately following the increase in bed shear stress. TSS concentrations increased by 161 and 206 % immediately after resuspension during Experiments $BII_{a,b}$, respectively. Following the increase in shear stress, the TSS began to decrease according to first-order kinetics. The removal rate coefficients for Experiments $BII_{a,b}$ were 0.05 and $0.04\,h^{-1}$, respectively. Nevertheless, despite the similar first-order removal trend, the time period where suspended solids concentrations exceeded $10\,mg\,l^{-1}$ was extended by more than 24 h in comparison to Experiments $BI_{a,b}$. The influence of resuspension on COD is not that pronounced and cannot be easily differentiated between Experiments $BI_{a,b}$ and Experiments $BII_{a,b}$, as the values scatter to a certain extent. As larger particles are characterized by faster sedimentation rates (Cizek et al., 2008; Droppo et al., 2009), it is assumed that the slower-settling, smaller particles were the predominant factor, which enhanced FIB persistence in Experiments $BII_{a,b}$ (see SECTION 5.2.3).

In a parallel experiment (same method Experiments $BII_{a,b}$) an attempt was made to investigate the fate of DOC following TSS resuspension. In FIGURE 5.4 the chromatograms for both the water and suspended solids are presented.

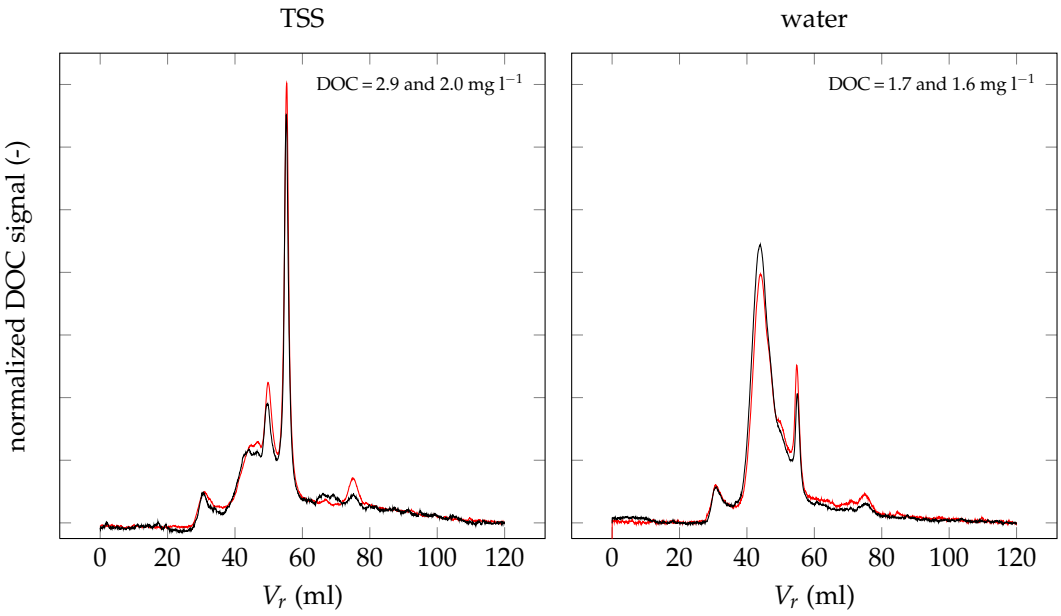


FIGURE 5.4: Normalized DOC measurements for the suspended solids and water at the beginning of the experiment (red curves) and after 21 h (black curves).

Here it can be seen that DOC eluted from the TSS contributed mainly to the low-weight fraction of DOC (V_r at 55 ml), which can be assumed to be more readily degradable. Contrary to this, the DOC associated with the water had a higher fraction of high-weight DOC (V_r between 35 and 55 ml), which is considered to be less readily degradable. This result suggests that the presence of TSS in the water column and continuous release of a low-weight fraction of DOC from it, could increase the persistence of suspended FIB.

5.2.3 Effect of increased TSS on the fate of FIB

To determine the impact of elevated TSS concentrations on FIB removal from the water column, the bulk phase was sampled over time and analyzed for *E. coli* and enterococci. The results for *E. coli* together with TSS are presented in FIGURE 5.5. Similar results were determined for enterococci, however for simplicity they have been left out of FIGURE 5.5.

The background concentrations in the water column (not shown) were at or below the method detection limit of 3.8×10^{-1} MPN ml^{-1}. Experiments $BII_{a,b}$ were characterized by similar trends. Initially, FIB concentrations increased by 3 - 4 orders of magnitude due to the CSO simulation after which they began to decrease. Immediately following the resuspension event when the discharge was increased from 0.1 to 0.2 m^3 s^{-1}, the removal rate of FIB was impacted. Based on the findings of Experiments $BI_{a,b}$, the initial in-store levels of FIB in the benthic biofilm were assumed to be relatively low, especially when compared to the FIB-laden water column. Thus, it came as no surprise that a spike in FIB concentrations in the water column following an abrupt increase in bed shear stress as reported by other groups (Jamieson et al., 2005b; Cho et al., 2010b) was not observed in these experiments. However, following the resuspension event the removal rate of FIB slowed considerably for a period of time, after which it again became more rapid. This effect of a delayed removal lasted approximately 20 h in both experiments.

In an attempt to more closely examine the difference in removal rates of *E. coli*, two first-order trend lines (black solid lines) have been inserted which differentiate between the slower and more rapid removal phases. Two additional first-order trend lines have been drawn which represent the standard removal rate of 0.2 h^{-1} (thick dark gray dashed line) as determined in Experiments $BI_{a,b}$ as well as the respective TSS removal rate coefficients of 0.05 or 0.04 h^{-1} (thick black dotted-dashed line) as determined in Experiments $BII_{a,b}$, respectively.

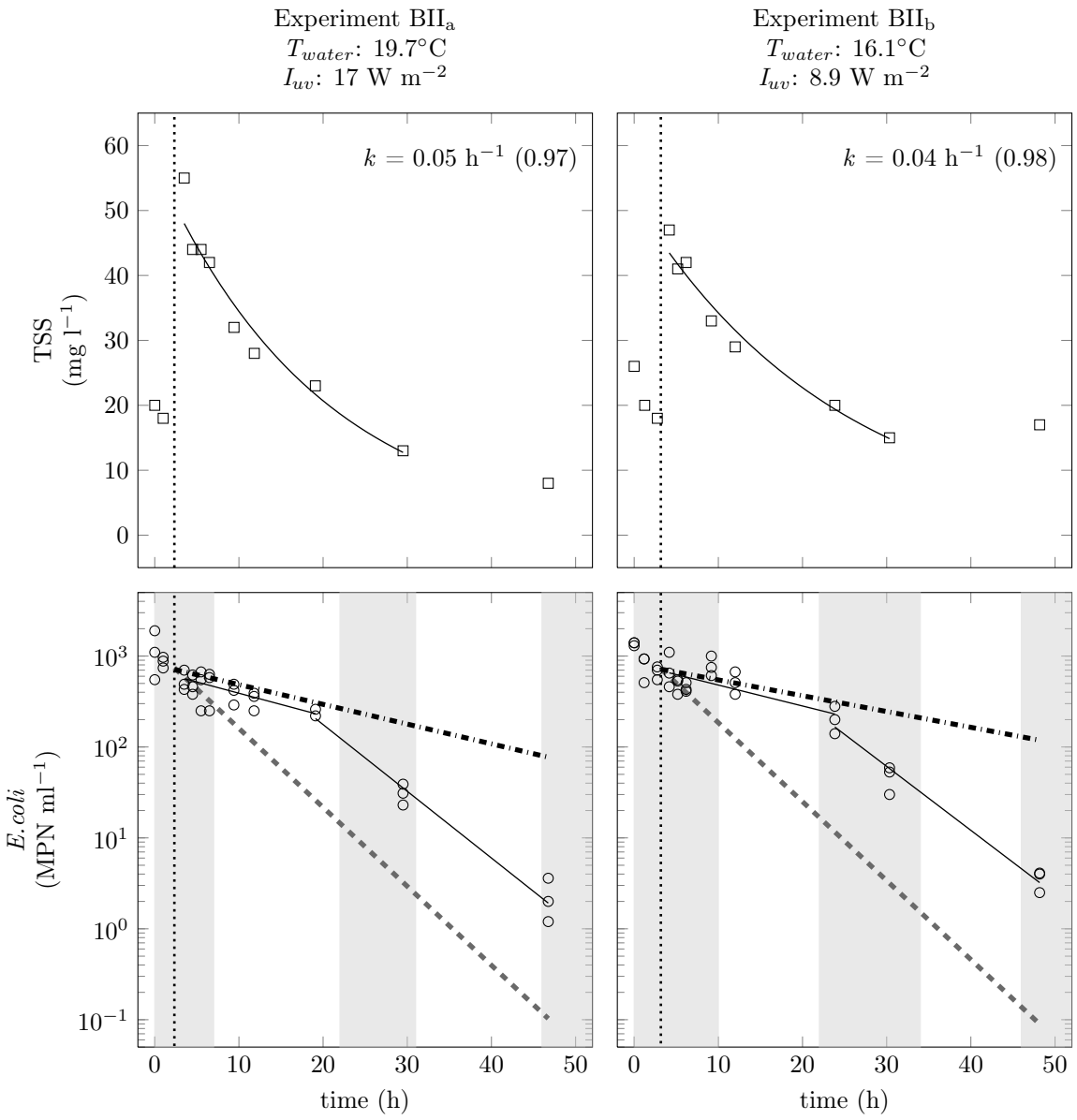


FIGURE 5.5: TSS and *E. coli* concentrations in the water column during Experiments BII$_{a,b}$. Dotted lines indicate when the flow rate was increased. Black solid lines depict first-order trend lines. Dark gray dashed lines represent first-order removal with a rate coefficient of 0.2 h^{-1}; black dotted-dashed lines are first-order removal trend lines with the same rate coefficient as TSS (0.05 or 0.04 h^{-1}).

Initially it was hypothesized that immediately following the resuspension event, when the flow rate had stabilized, *E. coli* concentrations would decrease at the same rate as determined in the standard experiments ($k = 0.2\,\mathrm{h}^{-1}$). This assumption was made based on the fact that following the resuspension event, the discharge was identical to that in Experiments BI$_{a,b}$. Additionally, UV inactivation in the flume system played only a small role in removal and thus, the increased turbidity should not have slowed removal. Moreover, deposition was shown to play an insignificant role in removal during Experiments BI$_{a,b}$. However, in FIGURE 5.5 it clearly can be seen that the concentrations do not follow this expected trend (dark gray dashed line), but rather

more closely adhere to the TSS removal rate (black dotted-dashed line) for approximately 20 h. In Experiment BII_a, this initial removal rate coefficient was $0.06\,h^{-1}$ ($R^2 = 0.62$) and in Experiment BI_b, a value of $0.05\,h^{-1}$ ($R^2 = 0.52$) was determined. The comparison presented in FIGURE 5.5 suggests that the elevated TSS levels increased the persistence of FIB in the water column. Again, as seen in FIGURE 5.4, the suspended solids contributed to a low-weight fraction of DOC which most likely was a contributing factor in the extended FIB persistence in the water column.

Although the fraction of particle-associated versus freely-suspended FIB was not specifically determined in this study, it is hypothesized that the elevated TSS concentrations led to an increased number of attachment sites for FIB. As a result, comparatively more FIB were associated with particles following the resuspension events than during the standard removal experiments (Experiments $BI_{a,b}$). Studies have shown that FIB preferentially attach to smaller particle sizes. For example, with a sieving and filtering technique, Auer and Niehaus (1993) investigated the association of fecal coliforms in an urban lake and determined that 90 % were attached to particles in the range of 0.45 - 10 µm. Behle (2011) examined the association of enterococci and *E. coli* in wastewater and found that more than 90 % were attached to particle sizes of 12 - 63 µm. It is hypothesized that due to this association with smaller particle sizes, FIB persistence in the water column was extended by nearly 20 h which thereby lengthened the time they were mobile in the environment.

Different research groups have attempted to elucidate the relationship between TSS and the association of FIB to particles. Soupir et al. (2010) explored the relationship between TSS and bacterial association in runoff from erodible soils. They observed a significant linear correlation between the attached fraction of enterococci in runoff from silty clay loam soil and TSS concentration. Additionally, Characklis et al. (2005) conducted studies to investigate microbial partitioning behavior in urban streams in North Carolina. They identified a positive relationship between the number of fecal coliforms attached to particles and particle concentrations.

Association with particulate matter provides microorganisms with advantages that they do not have when freely suspended. For example, it can offer them greater protection from environmental stressors such as predators or harmful ultraviolet light rays from the sun. Moreover, microorganisms may have greater access to nutrients, as the particles themselves can act as a food source. From TABLE 2.1, we know that 75 % of the fine bed sediment grain sizes were $\leq 20\,\mu m$. Moreover, the bed shear stress ($\approx 9\,N\,m^{-2}$) applied was well above the critical shear stress required to resuspend such fine sediments. Thus, we are of the opinion that due to the presence of these slower-settling, smaller particles in the water column, FIB persistence was enhanced and was characterized by the slower TSS removal rate. During this roughly 20 h period where FIB removal was influenced by TSS, only 50 % of the FIB were removed. As a comparison, during the first 20 h of Experiments $BI_{a,b}$ conducted at significantly lower

TSS concentrations, 95 % or more of the FIB were removed. As TSS concentrations in the Isar River during storm events can be well above 100 mg l^{-1}, the persistence of FIB introduced during a CSO could be significantly greater. As a result, they can travel longer distances in the river and thus, impact a much larger area.

After the period of slower removal evident in FIGURE 5.5, *E. coli* removal rates increased and were approximated by rate coefficients of 0.17 h^{-1} ($R^2 = 0.97$) and 0.16 h^{-1} ($R^2 = 0.96$), for Experiments BII$_{a,b}$, respectively. These values adhered very closely to the standard removal rate from the water column as determined in Experiments BI$_{a,b}$ ($k = 0.2\,\mathrm{h}^{-1}$; dark gray dashed line). Noteworthy is the fact that the removal rates increased in both experiments at the point where TSS levels were approximately 20 mg l^{-1}, or roughly the same TSS concentration as at the start of Experiments BI$_{a,b}$. Thus, it appears that after a certain period of time in a hostile environment such as an alpine oligotrophic river, FIB rapidly lose the ability to be cultured. In Experiments BI$_{a,b}$ this point was reached quicker; in Experiments BII$_{a,b}$, due to the higher TSS concentrations and the extended period of time in which they were present in the water column, this point occurred later.

Results from the two sets of experiments (BI$_{a,b}$ and BII$_{a,b}$) indicate that a sudden spike in the TSS levels (from approximately 20 to 50 mg l^{-1}) increases the persistence of FIB in the water column. However, when the TSS concentration decreases back to roughly 20 mg l^{-1}, a marked increase is seen in FIB removal rates. In further resuspension experiments, it would be of great interest to monitor the fraction of particle-associated versus freely-suspended FIB over time. Additionally, the removal behavior at higher TSS levels should be investigated. Finally, as deposition appears to play a role in FIB removal from the water column at higher TSS concentrations, more extensive monitoring of the riverbed is imperative.

5.3 Summary

- Large-scale flume systems represent an excellent tool for investigating the impacts of CSOs as they provide more realistic conditions than a lab-scale system, yet still offer a degree of control not attainable in rivers. For example, FIB removal rate coefficients determined in the water column of a large-scale flume were markedly higher than those of several studies found in literature which did not attempt to use realistic hydrodynamic conditions.

- In an oligotrophic river characterized by low TSS concentrations ($\approx 20\,\mathrm{mg\,l}^{-1}$), deposition of FIB onto the riverbed is insignificant. The majority of inactivation occurs in the water column.

- Particulate matter in water often results in lower inactivation rates of microor-

ganisms due to shielding of harmful UV rays. As UV inactivation played a lesser role in overall FIB removal in large-scale Flume B, it was possible to more precisely investigate the impact that high TSS levels have on FIB persistence. It was seen that at higher TSS loads ($\approx 50\,\mathrm{mg\,l^{-1}}$), FIB persistence in the water column was significantly enhanced, thus indicating the potential for more widespread microbial pollution.

- Despite the benefits that particle-association can offer FIB, after an extended period of time in a stressful environment a loss of their culturability ensues.

Chapter 6

The Effect of Particle-Association on UV Inactivation of Fecal Indicator Bacteria in Urban Rivers

The presence of particulate matter in water is known to hinder the transmission of UV light and therefore, leads to lower UV inactivation rates of 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 (Whitby and Palmateer, 1993; Örmeci and Linden, 2002). However, the degree to which particle association and particle size can impact UV inactivation of enteric microorganisms in dynamic 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 waterbodies 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 this chapter, the results of three groups of experiments are presented which help to clarify particle attachment and size on UV inactivation of FIB in river water following a CSO.

6.1 Impact of TSS on UV light penetration in the water column (Experiment CI)

In order to asses the effect of suspended solids on UV attenuation in the Obernach River, heavily TSS-laden water ($162\,\mathrm{mg\,l^{-1}}$) was collected and UV attenuation experiments were performed at different dilutions according to the procedure described in SECTION 2.6.3. In FIGURE 6.1, the vertical light attenuation coefficients obtained have been plotted against their respective TSS concentrations. A direct relationship between the two parameters was seen that could be described by the function: $k_{att} = 0.13\mathrm{TSS} + 0.27$ ($R^2 = 0.99$). In a study by Smith et al. (1999), the authors performed experimental work to investigate the main causes of attenuation of UV light in a lake

characterized by low dissolved organic matter concentrations. They found that TSS concentrations were the most useful water quality parameter for predicting UVB and UVA light attenuation. Smith et al. (1999) also provided linear regression models to predict UV attenuation coefficients, which were characterized by slopes ranging between 0.2 and 0.3. The value of 0.13 as determined in this study aligns well with this range.

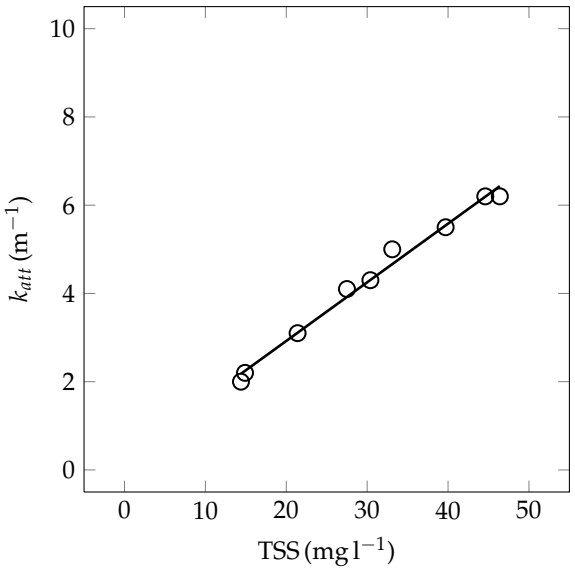


FIGURE 6.1: Effect of TSS on the vertical attenuation coefficient (k_{att}; EQUATION 2.6), described by the function: $k_{att} = 0.13\text{TSS} + 0.27$ ($R^2 = 0.99$).

During heavy rain events, TSS concentrations in the Isar and Obernach Rivers can increase to levels well over $100\,mg\,l^{-1}$. As seen in FIGURE 6.1, at a TSS concentration of roughy $50\,mg\,l^{-1}$, k_{att} is close to $6\,m^{-1}$. Considering Beer's Law (EQUATION 2.6), roughly only 5 % of the incoming UV light ($I_{290-390nm}$) can actually penetrate to a depth of 50 cm. At a water depth of 25 cm, nearly 75 % of the light is already attenuated. This indicates that UV inactivation of FIB primarily occurs in the top 25 cm of the water column. Moreover, any FIB which make it to the benthic substrata should remain protected from UV inactivation.

6.2 Wastewater characterization (Experiment CII)

Municipal wastewater is a critical component of stormwater overflow and thus, requires detailed characterization so as to better understand the fate and transport of enteric microorganisms in rivers. As part of this study, laser diffraction analyses were performed to measure the PSD of 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 6.1) and is discussed in the following. Re-

sults are from wastewater samples collected during dry periods to avoid any influence of rainwater.

TABLE 6.1: Particle size distribution measurements of municipal wastewater and contribution of the different fractions to FIB and TSS loading.

particle fraction (µm)	distribution in wastewater (%)	fraction *E. coli* adsorbed (%)	fraction enterococci adsorbed (%)	TSS contribution (%)	VSS (%)
$d_p \leq 12$	26	90.6	83.0	6	59
$12 < d_p \leq 63$	27	8.6	16.3	45	75
$63 < d_p \leq 1000$	33	0.6	0.6	23	88
$d_p > 1000$	14	0.2	0.1	26	89

6.2.1 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 $63 < d_p \leq 1000\,\mu m$ and $d_p > 1000\,\mu m$ 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 $d_p > 1000\,\mu m$ it was close to 90 %.

6.2.2 Microbial fractionation

Sieve and filter residues were additionally analyzed for their respective FIB concentrations. 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 $63 < d_p \leq 1000\,\mu m$, containing 8.6 % of *E. coli* and 16.3 % of all enterococci (see TABLE 6.1). 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 and protection from sunlight.

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 SECTION 6.2.1 and SECTION 6.2.2 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; de Brauwere et al., 2011; Gao 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.

6.3 UV inactivation of FIB associated with different particle size fractions (Experiments $CIII_{a,b,c}$)

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 ($I_{290-390nm} = 8.0\,W\,m^{-2}$). Results for the first 8 h of each experiment are presented in FIGURE 6.2. 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 6.2 the k-values have been summarized; the influence of TSS and particle size on the inactivation rates will be discussed in the following sections.

TABLE 6.2: Summarized removal rate coefficients for *E. coli* and enterococci with respect to particle size fractions as determined in Experiments $CIII_{a,b,c}$.

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)
$d_p > 1000$	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)

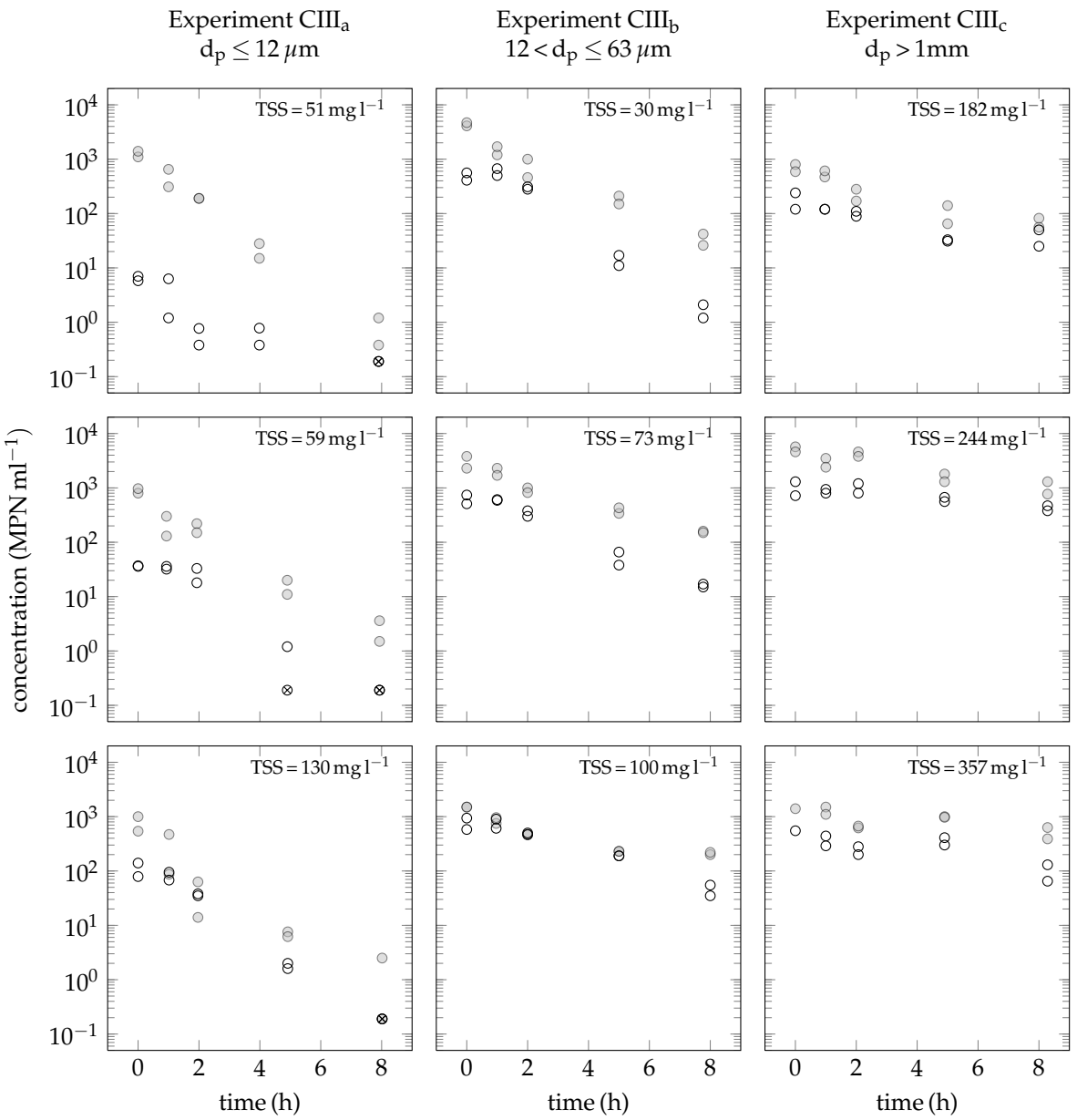


FIGURE 6.2: Measured *E. coli* and enterococci concentrations for different particle fractions during the first 8 h of each of three groups of experiments (Experiments CIII$_{a,b,c}$). The corresponding TSS concentration is noted in all subplots. Shaded circles represent *E. coli* and white circles enterococci. ⊗ indicates that concentrations were no longer detected.

6.3.1 Impact of TSS on UV inactivation rates

From TABLE 6.2, it can be seen that TSS concentrations differed between experiments and that for $d_p > 1000\,\mu m$, 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 6.1) 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 $d_p > 1000\,\mu m$ (TABLE 6.2), UV light did not impact the inactivation rates. However, considering the fractions $d_p \leq 12\,\mu m$ and $12 < d_p \leq 63\,\mu m$, 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 (Pommepuy et al., 1992; Burkhardt III et al., 2000; Chigbu et al., 2005; Sinton et al., 2007; Schultz-Fademrecht et al., 2008), this pronounced reduction in k at higher TSS concentrations can result in a larger geographical region being impacted by fecal contamination following a CSO.

6.3.2 Influence of particle size on UV inactivation rates

In addition to the impact of TSS concentrations on FIB removal rate coefficients, the particle size fraction with which FIB were associated with also influenced k-values. This effect was most evident for the fractions $d_p \leq 12\,\mu m$ and $12 < d_p \leq 63\,\mu m$, where TSS concentrations were in a similar range. As seen in FIGURE 6.3 and TABLE 6.2, 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 $d_p > 1000\,\mu m$ 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, they found that the fewer, but larger particles still provided more protection.

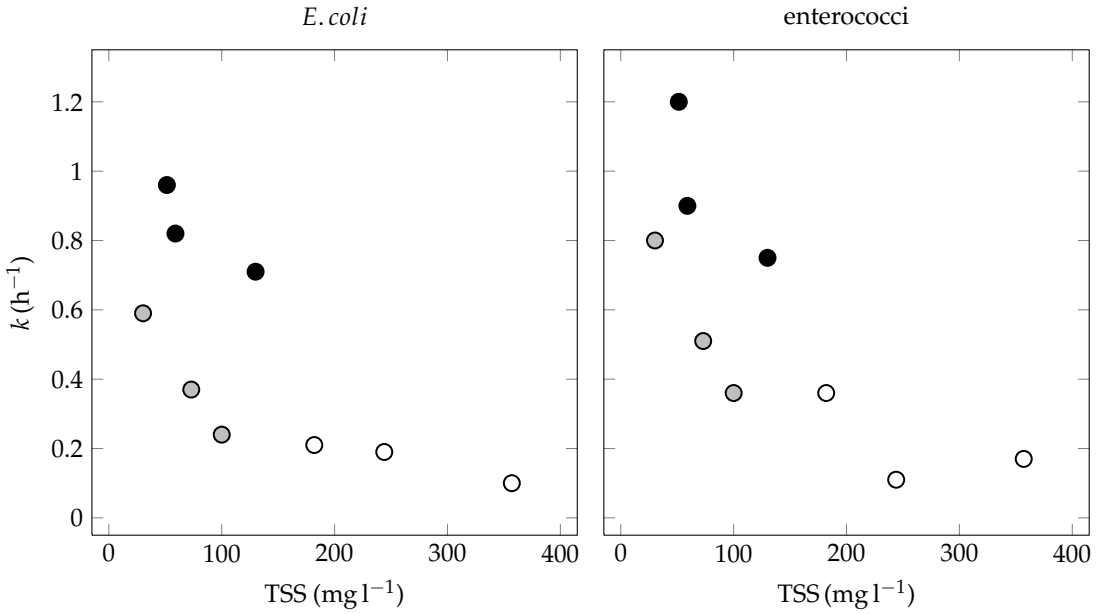


FIGURE 6.3: Removal rate coefficients for $d_p \leq 12\,\mu m$ (black, Experiment $CIII_a$), $12 < d_p \leq 63\,\mu m$ (gray, Experiment $CIII_b$), and $d_p > 1000\,\mu m$ (white, Experiment $CIII_c$) depicted against their respective TSS concentrations.

From the data presented in TABLE 6.1, 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 $d_p \leq 12\,\mu m$. 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 $12 < d_p \leq 63\,\mu m$ and $d_p > 1000\,\mu m$, 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.

6.3.3 Impact of dark and light conditions on FIB inactivation

In FIGURE 6.4, the effect of UV light ($I_{290-390nm}$) 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 $I_{290-390nm}$ 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 6.3.1 and 6.3.2, 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 ($I_{290-390nm} = 0.0\,W\,m^{-2}$).

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

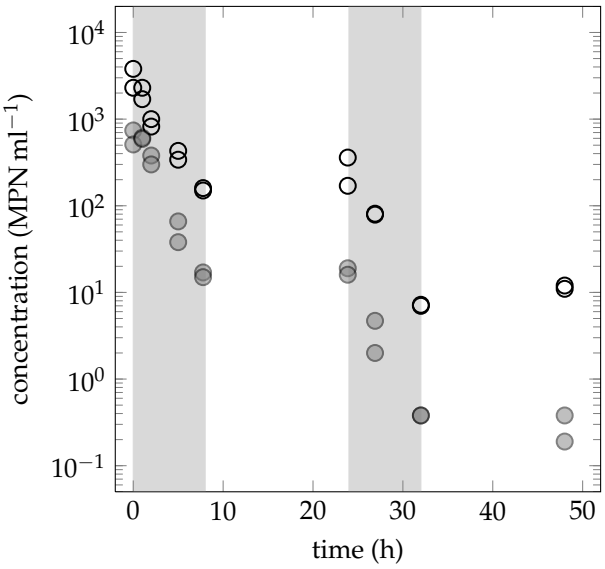


FIGURE 6.4: UV inactivation pattern of *E. coli* (white) and enterococci (gray) attached to the particle fraction $12 < d_p \leq 63\mu m$ throughout one complete experiment. The TSS concentration was 73 mg l^{-1}. Shaded regions indicate when UV lamps were on.

however, how much should read into this stagnation in FIB removal when $I_{290-390nm} = 0.0\,W\,m^{-2}$. As these experiments 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.

6.4 Summary

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, the impact of TSS concentrations on UV light attenuation was investigated. Subsequently, particle size distribution measurements were made of municipal wastewater. Finally, 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 have been drawn:

- At a TSS concentration of approximately 50 mg l^{-1}, nearly 80 % of UV light is attenuated within the first 25 cm of the water column. This indicates that the majority of UV inactivation of FIB occurs in the top 25 cm. Moreover, should FIB wind up in the benthic zone, UV inactivation no longer plays a large threat to

their survival.

- 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 $12 < d_p \leq 63\,\mu m$.

Chapter 7

Conclusions

This work set out to examine the threat combined sewer overflows can pose to an oligotrophic, alpine river. The microorganisms *E. coli* and enterococci were selected as target indicators of fecal contamination based on their widespread usage in the field of water quality monitoring and assessment. In both lab-scale and large-scale flumes, experiments were conducted to investigate different processes influencing microbial water quality, including deposition, inactivation, and resuspension. Moreover, the influence of suspended solids on FIB inactivation as well as the persistence and potential for FIB growth in streambed sediments was evaluated under different bed shear stresses. The results have been summarized in TABLE 7.1 (page 89) and will be highlighted briefly in the following.

7.1 Removal of FIB from the water column

For years, the impact of sewer overflows on the microbial quality of surface waters has been studied. Researchers have worked at a wide variety of scales, ranging from small batch experiments to *in situ* measurements. However, a significant amount of work looking at the fate and transport of FIB in the water column of streams has been done in lab set-ups, where more emphasis has been placed on mimicking the biological factors rather than the hydrodynamic conditions.

In this work, both lab and large-scale flumes were used to gain a better understanding of the fate and transport of FIB in the water column of the Isar River. Interestingly, a significant difference was observed between FIB persistence in the water columns of the two systems. In the small-scale flume, removal consistently occurred four times slower than in the large flume. Although TSS concentrations in the two systems were not identical (roughly $10\,\mathrm{mg\,l^{-1}}$ lower in lab flume), COD concentrations as well as temperature and pH were very similar. Moreover, the depth:length ratios and residence times in the flumes were comparable, however only in the large-scale flume was it possible to conduct experiments under turbulent conditions. All of this suggests that factors such as turbulence or being able to operate under more natural conditions led

to the differing removal patterns. The question is thus raised whether lab-scale flumes can accurately mimic the behavior of enteric pollution in streams.

In addition, an attempt was made to examine the individual contributions of natural inactivation and deposition on overall FIB removal from the water column of the lab-scale flume. Here it was seen that at TSS concentrations of roughly $10\,mg\,l^{-1}$, close to 85 % of the removal observed in the first 24 h could be attributed to natural inactivation, most likely the result of the low water temperature and nutrient levels. These findings would suggest, that deposition of FIB to the streambed is minimal and thus, the formation of pathogen reservoirs in bed sediments should be limited. However as already noted, FIB removal from the water column occurred at very different rates in the two different systems. Therefore, extrapolating these results and blindly applying them at the large-scale is not advised.

7.2 Influence of suspended solids on FIB persistence

Understanding the impact that suspended solids have on the microbial quality of surface waters is paramount. From literature it is known that UV light is perhaps the most significant factor contributing to FIB inactivation in shallow surface waterbodies. Moreover, from experience in water and wastewater treatment practices it is known that the presence of particulate matter can appreciably reduce the efficacy of UV disinfection. Through a series of column experiments performed with natural river water and suspended bed sediments, it was seen that already at a TSS concentration of $50\,mg\,l^{-1}$, close to 80 % of UV light (290 - 390 nm) was attenuated in the top 25 cm of the water column. This suggests that the principal FIB inactivation process is only applicable to the uppermost region of the water column. Should enteric microorganisms be transported below this point, their chances of survival would drastically improve. As water depths in the Isar River often exceed 1 m in the Munich region, it is essential to consider the role suspended solids have on microbial water quality.

After characterizing the municipal wastewater used in this study, it was seen that the majority of *E. coli* (90.6 %) and enterococci (83.0 %) were found within the size fraction $d_p \leq 12\,\mu m$. In experiments performed to compare UV inactivation of FIB attached with different particle sizes, it was noted that their removal from the water column occurred approximately two times more rapidly when attached to the fraction $d_p \leq 12\,\mu m$ than when found in the range $12 < d_p \leq 63\,\mu m$. Although the majority of FIB in wastewater are associated with the fraction marked by rapid UV inactivation rates, should significant light attenuation occur due to elevated TSS concentrations, microbial water quality will be characterized by the transport of this fraction.

Not only does particulate matter in the water column shield harmful UV rays, it can also act as a food source and mode of transport for enteric microorganisms. Through

a group of experiments conducted in a large-scale flume, the impact of TSS on the persistence of FIB in the water column was studied. Here it was seen that FIB removal from the water column was four times slower at TSS concentrations above $20\,\mathrm{mg\,l^{-1}}$. During intense rain events, suspended sediment concentrations in the Isar River can well surpass the $100\,\mathrm{mg\,l^{-1}}$ mark. This fact together with the findings suggest that during such events, the risk of more widespread enteric pollution is elevated due to higher suspended solids concentrations.

7.3 Fate of FIB in streambed sediments

The ability to accurately predict microbial water quality requires a sound understanding and characterization of all possible sources and sinks in a watershed. One environmental compartment which has proven tricky to approximate is the benthic zone. In comparison to the bulk phase, this region is often marked by higher nutrient levels, limited UV light penetration, and more favorable hydrodynamic conditions, all of which can promote FIB persistence. Although in literature it has been repeatedly shown that bed sediments can promote persistence and even lead to growth of enteric pathogens, few studies undertaken have attempted to investigate survival under realistic hydrodynamic conditions.

As part of this study, experimental work was performed with the sediments of an oligotrophic river to investigate the impacts of bed shear stress and sediment depth on the survival of FIB. Under ideal conditions (*e.g.* no UV light, no grazers/predators, low shear stresses), *E. coli* persisted twice as long as enterococci, irrespective of whether a thick (cm) layer of sediment was used or a thin (µm) biofilm. However, as soon as pressure from grazers/predators was introduced into the system characterized by a more realistic bed shear stress, the two FIB were eliminated at a similar, but more rapid rate. Moreover, a thicker layer of sediment was capable of harboring FIB longer than the thin biofilm. These findings indicate that batch experiments, conducted without flow or under unrealistically low bed shear stresses in the absence of grazers/predators, may provide an inaccurate depiction of what actually occurs in natural stream systems.

7.4 Outlook

Although the topic of enteric pollution of surface waters has been of interest for decades and considerable work has been done to better predict their fate and transport within the aquatic environment, there are still areas of uncertainty clouding the topic. As increasingly complex mathematical models are created to more accurately assist regu-

lators in the decision-making process, more exact data is in turn also required for their calibration and validation.

Knowing that suspended solids can enhance FIB survival in the water column has prompted modelers to incorporate terms to account for the fraction of FIB which are particle-associated versus freely-suspended. Despite the fact that different methods have been used to investigate attachment of FIB to particulate matter (*e.g.* centrifugation or fractional filtration), no single technique has proven ideal. Efforts should be placed on designing a new method or honing a current technique so that precise data of particle size and degree of FIB association can be ascertained. Furthermore, once the magnitude of association is estimated, attempts at determining FIB-specific deposition rates under different hydrodynamic conditions can be made.

Diminished water quality with respect to enteric pollution has been linked with the re-entrainment of bed sediment due to an increase in the bed shear stress or physical disruption of bed sediments by bathers. In this study it was seen that resuspension events can prolong the persistence of FIB in the water column due to increased suspended sediment concentrations. Further studies should not simply aim at monitoring FIB persistence, but also more closely analyze the particle size fractions to which FIB are attached as well as the fractions of dissolved organic carbon associated with the bulk phase and suspended solids. Gaining more insight into the types of organic carbon molecules that the water phase and suspended sediments can offer enteric microorganisms, may help clarify the relation between TSS and FIB persistence. The SEC-DOC method is a promising technique which can provide information regarding the sizes of organic carbon molecules available in different samples.

As demonstrated in this work, only minimal success was achieved in simulating natural river conditions in a lab setting. As striking differences were seen in FIB persistence in both the water columns and bed sediments of the lab and large-scale flumes, future research should be done in systems which can realistically mimic stream hydraulics.

TABLE 7.1: Summarized removal rate coefficients determined in the water column and benthic sediments.

flume system	phase	overall *E. coli* inactivation (k_{ec})	overall enterococci inactivation (k_{ent})	removal due to natural inactivation ($k_{I,FIB}$)	removal due to deposition ($k_{D,FIB}$)
lab-scale	water ($\approx 10\,mg_{TSS}\,l^{-1}$)	$0.05\,h^{-1}$	$0.06\,h^{-1}$	83 %	17 %
large-scale	water ($\approx 20\,mg_{TSS}\,l^{-1}$)	$0.2\,h^{-1}$	$0.2\,h^{-1}$	n.d.	n.d.
large-scale	water ($\approx 50\,mg_{TSS}\,l^{-1}$)	$0.06\,h^{-1}$	$0.06\,h^{-1}$	n.d.	n.d.
lab-scale	sediment	$0.6-0.8\,d^{-1}$	$0.3-0.4\,d^{-1}$	n.d.	n.a.
large-scale	sediment	$0.8-1.2\,d^{-1}$	$0.7-1.5\,d^{-1}$	n.d.	n.a.

*n.d. = not determined; n.a. = not applicable

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