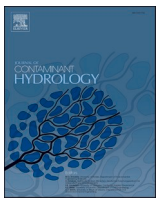
Journal of Contaminant Hydrology 251 (2022) 104080

Contents lists available at ScienceDirect

Journal of Contaminant Hydrology

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



Transport and removal of spores of *Bacillus subtilis* in an alluvial gravel aquifer at varying flow rates and implications for setback distances

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ARTICLE INFO

Keywords:
Riverbank filtration
Tracer test
QMRA
Safe setback distance
Cryptosporidium
Campylobacter

ABSTRACT

To guarantee proper protection from fecally transmitted pathogen infections, drinking water wells should have a sufficiently large setback distance from potential sources of contamination, e.g. a nearby river. The aim of this study was to provide insight in regards to microbial contamination of groundwater under different flow velocities, which can vary over time due to changes in river stage, season or pumping rate. The effects of these changes, and how they affect removal parameters, are not completely understood. In this study, field tracer tests were carried out in a sandy gravel aquifer near Vienna, Austria to evaluate the ability of subsurface media to attenuate *Bacillus subtilis* spores, used as a surrogate for *Cryptosporidium* and *Campylobacter*. The hydraulic gradient between injection and extraction was controlled by changing the pumping rate (1, 10 l/s) of a pumping well at the test site, building upon previously published work in which tracer tests with a 5 l/s pumping rate were carried out. Attachment and detachment rate coefficients were determined using a HYDRUS-3D model and ranged from 0.12 to 0.76 and 0–0.0013 h^{-1}, respectively. Setback distances were calculated based on the 60-day travel time, as well as a quantitative microbial risk assessment (QMRA) approach, which showed similar results at this site; around 700 m at the highest pumping rate. Removal rates (λ) in the field tests ranged from 0.2 to 0.3 log/m, with lower pumping rates leading to higher removal. It was shown that scale must be taken into consideration when determining λ for the calculation of safe setback distances.

1. Introduction

Waterborne disease outbreaks are still a major health issue worldwide (Beer et al., 2015; Beller, 1997; Rasmuson et al., 2019). To reduce the risk of these outbreaks, pre-treatment steps such as riverbank filtration can be used to lower pathogen concentration (Ray et al., 2002; Sharma et al., 2012). During subsurface flow, the removal of pathogens is influenced by groundwater flow rate, which can vary due to factors such as groundwater abstraction, river stage, seasonal changes, city planning decisions (such as stream rehabilitation) and effects of climate change. Furthermore, water level fluctuations can lead to higher concentrations of pathogens (Derx et al., 2013).

In many countries, an adequate removal of pathogens is usually assumed when subsurface travel times are 60 days or more, and setback distances for drinking water wells are often calculated on this basis (Schijven and Hassanizadeh, 2002). However, in contrast to this 60-day time of travel (TOT) approach, the World Health Organization (WHO) has recommended that a quantitative microbial risk assessment (QMRA) approach should be used for defining safe setback distances for drinking water production (WHO, 2011, 2017). To be able to carry out a QMRA, reliable transport and removal parameters describing subsurface flow are needed. To accurately quantify these parameters, tracer tests should be carried out at the site of interest, using surrogates that experience similar transport and removal as pathogenic microorganisms.

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https://doi.org/10.1016/j.jconhyd.2022.104080
Received 5 April 2022; Received in revised form 29 July 2022; Accepted 17 September 2022
Available online 23 September 2022

Studies in columns show that faster flow rates can lead to less removal (Choi et al., 2007; Ryan and Gschwend, 1994; Walshe et al., 2010), and attachment and detachment rates have been shown to vary with decreasing pore velocity (Bradford et al., 2006; Hendry et al., 1999). An explanation for this is that lower velocity leads to longer residence times, which increase the probability of microbes to diffuse over the energy barrier, as well as leading to lower hydrodynamic forces, decreasing detachment (Sasidharan et al., 2017). Furthermore, Colloid Filtration Theory (CFT) predicts that the attachment efficiency (α) is dependent on the single-collector contact efficiency (η_0), which in turn depends on the flow velocity, because it affects, among other processes, the particle deposition rate due to interception (Tufenkji and Elimelech, 2004; Yao et al., 1971). This shows that velocity is an important factor affecting bacterial removal in groundwater. However, most tracer tests in the field are carried out within natural (i.e. unforced) gradient flow conditions, which makes it difficult to observe the effects of different flow rates on microbial removal directly (DeBorde et al., 1999; Mallén et al., 2005; Pang et al., 2005). Comparing removal rates at different flow velocities is usually done by comparing tests in different aquifers, and few tests have been carried out with varying flow rates at the same test site (Kvitsand et al., 2015; Pang, 2009). Therefore, it is not well understood at the field scale if and how changes in flow velocity affect microbial removal and the parameters that govern it.

In order to compare setback distances at the various flow rates, a QMRA was carried out for *Cryptosporidium* and *Campylobacter*, as well as calculations of the traditional 60-day TOT. Both pathogens considered in this study are commonly found in human and animal waste, and can cause gastrointestinal illnesses leading to severe and prolonged diarrhea, which might pose significant health risks, especially for vulnerable and immunocompromised patients (Hoogenboezem et al., 2001; Percival and Williams, 2013; Teunis et al., 2005; Teunis et al., 2002; WHO, 2011). *Campylobacter* are fecally borne bacteria that have led to numerous waterborne disease outbreaks in the past years, even in developed countries (Craun, 2012; Guzman-Herrador et al., 2015). *Cryptosporidium* are protozoa, usually present in the form of oocysts which resist degradation, and are generally more persistent in the environment, not unlike spores of *B. subtilis* (Headd and Bradford, 2016). *Campylobacter* is removed more readily, as it is not spore- or oocyst-forming and therefore experiences more die-off and inactivation, processes whereby the organism either dies or is unable to infect (Schijven et al., 2013).

The groundwater flow rates in this study were controlled by changing the pumping rate of an abstraction well in the area, where tracer tests were carried out using spores of *Bacillus subtilis*. These endospore-forming bacteria are very persistent during transport and are therefore regarded as worst-case scenario microbial tracers to study the removal of bacteria in the subsurface (Li et al., 2018; Pang et al., 1998; Setlow, 1995). *B. subtilis* (~1.5 μm long) is of similar size when compared to many important bacterial pathogens such as *Salmonella* spp. and *Campylobacter* spp., and is also used as a surrogate for *Cryptosporidium* spp. (which is up to 7 μm long), even though there are differences in size, surface charge and hydrophobicity (Bradford et al., 2016a; Emelko and Huck, 2004; Cools et al., 2003); Chen et al., 2010).

This study builds upon a previous study by Oudega et al. (2021), by performing additional field tracer tests at different pumping rates, which are modeled in HYDRUS-3D to attain reliable parameters for the assessment of alluvial aquifers. Furthermore, this study adds a comparison of the 60-day TOT versus the QMRA approach for defining the setback distances from a drinking water well, using *Cryptosporidium* and *Campylobacter* as reference pathogens.

2. Materials and methods

2.1. Field site

The study was carried out at the Obere Lobau test site located near the River Danube in Vienna, Austria, and contains alluvial sediments of mostly gravel and sand (d_{50} = 4 mm, C_U = 38.4). The effective porosity of the material is 0.12 and the hydraulic conductivity is $7.5 \bullet 10^{-3}$ m/s. The groundwater at the site is not in direct contact with Danube river water due to the presence of a dam, and contains little to no oxygen, a near-neutral pH and a high iron content. A more extensive description of the study site, sediment and groundwater characteristics is given in Oudega et al. (2021). The site contains an injection well (P24) and a pumping well (LB13) at a distance of 25 m, which also serves as the sampling point (Fig. 1). There are additional pumping wells north and south of the study site, which together create a drawdown of hydraulic head and a predominantly west to east groundwater flow. The injection well has a well screen from 8 to 14 m depth, the pumping well has a well screen from 5 to 23 m depth, and both well screens are located fully in the saturated zone.

2.2. Experimental design

To allow for direct comparison of transport processes at different flow rates, duplicate tracer tests were performed in a forced gradient system with pumping rates of 1, 5 and 10 l/s (Table 1). These pumping rates are about an order of magnitude lower than the maximum extraction rates in drinking water wells downstream of the study site. However, due to regulations, higher pumping rates were not allowed. The flow rates given in this table are averages over the transport distance, but not uniform, as they increase towards the pumping well. All experiments were done with spores of *B. subtilis* and a conservative

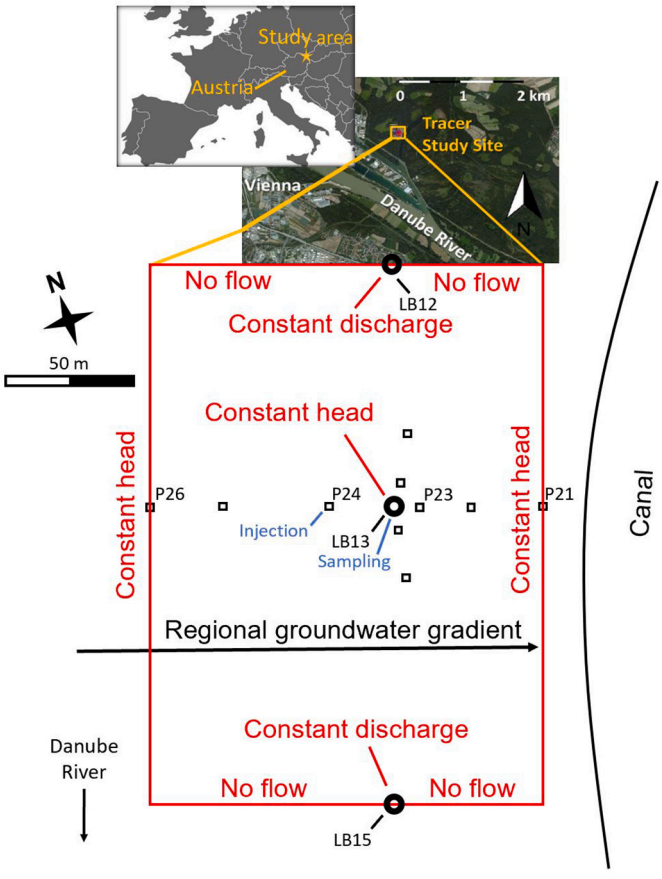

Fig. 1. Location of the tracer studies conducted in 2018 and 2019. The model domain and boundary conditions are shown in red. Circles (LB) are pumping wells, squares (P) are piezometers. The map of the boundary conditions is taken from Oudega et al. (2021), in which the modeling is described in more detail. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Field experiments carried out with spores of *B. subtilis*.

Test	Date	Pumping Rate (l/s)	Gradient (−)	Average Flow rate (m/h)	Conservative Tracer	*B. subtilis* injected (CFU/ml)
1pre	06.12.18	1.09	0.0045	0.102	Both	–
1a	07.01.19	1.20	0.0023	0.098	Uranine	1.33×10^9
1b	18.02.19	0.99	0.0028	0.079	Uranine	9.13×10^8
5a[a]	24.04.18	5.05	0.0194	0.615	Bromide	1.10×10^9
5b[a]	28.05.18	4.65	0.0189	0.557	Bromide	7.73×10^8
10a	21.01.19	9.57	0.0311	1.015	Uranine	5.64×10^8
10b	28.01.19	9.45	0.0318	0.977	Uranine	7.87×10^8

[a] Published in Oudega et al. (2021).

tracer; either uranine or bromide. Tests 5a-b were done with bromide using a pumping rate of 5 l/s (results published in Oudega et al., 2021). The additional tests for this study were carried out with uranine: tests 1a-b with a 1 l/s pumping rate and tests 10a-b with a 10 l/s pumping rate. Test 1pre was carried out with both bromide and uranine to check whether the transport behavior of the two conservative tracers was comparable to one another. The absolute difference in gradient between duplicate experiments was never >2.8‰.

2.3. Characteristics, culture and essay of the tracers

2.3.1. Bacillus subtilis

All tests were carried out with *B. subtilis* endospores (strain ATCC 6633), which are rod-shaped, aerobic and non-pathogenic bacteria present in low-temperature environments (Harden and Harris, 1953; Headd and Bradford, 2016; Wightman et al., 2001). In the spore state, *B. subtilis* bacteria are well protected against damage and survive for a long time (Setlow, 1995; Setlow and Johnson, 2019). Using an electron microscope (FEI Company, Hillsboro, USA), the spores were measured to be 1.5 μm in length and 0.5 μm in width. They have an isoelectric point of pH 2.2 and are strongly hydrophilic (Bradford et al., 2016b; Harden and Harris, 1953).

Preparation and assay was described in Oudega et al. (2021). The spores were injected in a concentration of approximately 10^9 spores/ml. The suspension of 1.5 l groundwater was injected in 1.5 min at 7 m depth by using a peristaltic pump.

Samples of 12 ml were taken by an autosampler from a flow-through cell linked to pumping well LB13, and stored in glass test-tubes. An initial interval of 5 min between samples was used in tests 5a-b and 10a-b, and 60 min in tests 1a-b. These intervals were extended after the peaks passed. Depending on the expected concentration, sample volumes of 1, 2, 3, 6 or 9 ml were used on one or up to three petri dishes. The lower the expected concentration, the higher was the sample volume analyzed, using the pour plating technique and PC-Agar (Merck, Darmstadt, Germany).

2.3.2. Bromide

According to the maximum concentration of 100 mg/l NaBr permitted by the government, the bromide (100 g) was injected in a volume of 1000 l groundwater to ensure sufficient concentration in the samples. The bromide solution was injected by fuel pump 2 min after the injection of the *B. subtilis* spore suspension and took approximately 15 min. We assumed no mixing between the two tracer solutions. The samples were analyzed at the TU Wien with HP/LC Chromatography (Metrohm ECO IC, Herisau, Switzerland).

2.3.3. Uranine

Uranine was used in tests 1a-b and 10a-b in a concentration of 10 g/l and a volume of 10 l groundwater, using the same injection method as for bromide. The fluorescence of the water was measured directly in the flow-through cell using a GGUN-FL24 flow-through field fluorospectrophotometer (Albillia Co, Neuchatel, Switzerland).

2.4. Modeling

2.4.1. Hydrus

The field tests were modeled using HYDRUS-3D software (Šimůnek et al., 2016). The three-dimensional domain was defined as a cuboid with a depth of 24 m, which is the top of a clayey aquitard in the study area. Constant heads were assigned to the domain boundaries upstream and downstream as well as on the main pumping well, as to maintain perfect control over the hydraulic gradient (Fig. 1). No flow conditions were assigned to the north and south boundaries on either side of the flow line, except for two pumping wells on these boundaries, which were modeled by constant discharge. The values for hydraulic conductivity were found by calibration on the basis of the measured water levels in the piezometers during tests. The injection solution was assumed to be mixed in the entire well screen. Calibration of dispersivity values was done by fitting the modeled breakthrough curves (BTCs) of conservative tracers to the measured data. More details about the model and its parameters can be found in Oudega et al. (2021). To calculate the model-derived 60-day TOT distances, the modeled hydraulic gradient was extrapolated in space for each test. Darcy's equation was then employed, using the calibrated values for hydraulic conductivity and porosity.

The subsurface flow was simulated in 3 dimensions with the Richards' equation (Simunek et al., 2012):

$$\frac{\partial \theta}{\partial t} = \frac{\partial}{\partial x_i}\left[K(h)\left(K_{ij}^A \frac{\partial h}{\partial x_j} + K_{iz}^A\right)\right] \quad (1)$$

where θ is the water content (−), t is time (T), x_i is the spatial coordinate (L), K is the hydraulic conductivity (L/T), h is the pressure head (L) and K_{ij}^A are components of a dimensionless anisotropy tensor.

The transport of spores of *B. subtilis* was modeled using the following advection-dispersion equation and two-site attachment-detachment model (Schijven and Šimůnek, 2002):

$$\frac{\partial C}{\partial t} = D_x \frac{\partial^2 C}{\partial x^2} + D_y \frac{\partial^2 C}{\partial y^2} + D_z \frac{\partial^2 C}{\partial z^2} - v\frac{\partial C}{\partial x} - \frac{\rho_b}{\theta}\frac{\delta S_1}{\delta t} - \frac{\rho_b}{\theta}\frac{\delta S_2}{\delta t} \quad (2)$$

$$\frac{\rho_b}{\theta}\frac{\delta S_1}{\delta t} = k_{att1} C - \frac{\rho_b}{\theta} k_{det1} S_1 \quad (3)$$

$$\frac{\rho_b}{\theta}\frac{\delta S_2}{\delta t} = k_{att2} C - \frac{\rho_b}{\theta} k_{det2} S_2 \quad (4)$$

where C is equal to the concentration of free spores of *B. subtilis* (M/L^3), D is spatial dispersion (L^2/T), v is the pore-water velocity (L/T, whereby x is the direction of flow), ρ_b is bulk density (M/L^3) and S is the concentration of attached particles (M/L^3). Values for k_{att} and k_{det} (1/T), attachment and detachment rate coefficients, respectively, were found by calibrating the model to the BTC of each test.

2.4.2. QMRAspot

QMRAspot is a computational tool to analyze and conduct a quantitative microbial risk assessment (QMRA) for drinking water. It was used for this study site to calculate the necessary removal of *Cryptosporidium* oocysts and *Campylobacter* during subsurface transport. The

model uses Monte Carlo simulations to calculate the risk of infection, based on the distribution of the input data, as well pathogen-specific dose response parameters (Schijven et al., 2011). This is done in order to reach a health based target which is the criterion to minimize the risk of infection below 10^{-4}/person/year, based on recommendations by the WHO (WHO, 2011). To reach this target, QMRAspot was calibrated by trial and error for the necessary subsurface removal. The input parameters for the program are given in Table 2. The source concentrations of *Cryptosporidium* and *Campylobacter* were obtained from Demeter et al. (2021), who used a probabilistic-deterministic water quality model of the Danube, which considered the major wastewater sources upstream of the study site. This water quality model produced a mean and 95th percentile concentration for each pathogen, from which the program created a distribution to run 10.000 Monte Carlo simulations, resulting in a specific risk (infections/person/year). Two separate inactivation rates were used for *Campylobacter*, because of large differences in the literature. For *Cryptosporidium*, these differences were smaller and therefore of less influence on the resulting setback distances, which is why only one value was used. Dose-response curves for each pathogen are included in the program (Schijven et al., 2011; Teunis et al., 2005; Teunis et al., 2002).

2.5. Analytical methods

BTCs for the conservative tracers and *B. subtilis* were plotted as sample concentrations over time, normalized by the initial concentration. From this data, spatial removal rates λ (1/L) for *B. subtilis* spores were calculated for each test as per Eq. 5, which is valid for three dimensions if the flow rate is constant (Kretzschmar et al., 1997; Pang, 2009):

$$\lambda = -\frac{ln\left(\frac{Q}{N_0}\int_0^{t_f} C(t)dt\right)}{x} = -2.3\frac{log_{10}\left(\frac{Q}{N_0}\int_0^{t_f} C(t)dt\right)}{x} \quad (5)$$

where Q is the flow rate in the well (L^3/T), N_0 is the total amount of microbial tracer injected (M), C(t) the concentration at a given time, t, after injection (M/L^3), t_f is the final time of the test (T), and x the distance traveled to the pumping well (L). The integration was approximated by dividing the time series into sampling intervals, of which it was assumed that the sample concentration was an average value for that time interval.

The setback distance for the QMRA method was calculated by the 1D advection-dispersion equation coupled with the removal, λ, and inactivation rate (modified from Blaschke et al., 2016):

$$x = 2.3\,\frac{2\alpha_l log(F)}{1-\sqrt{1+4\alpha_l\left(\frac{\lambda\bullet v+\mu}{v}\right)}} \quad (6)$$

where F is calculated by QMRAspot and is the necessary removal of *Cryptosporidium* or *Campylobacter* by subsurface transport (as a fraction) to meet the health based target, α_l is the longitudinal dispersivity (L), and μ is the inactivation rate (1/T).

Table 2
Pathogen-specific input parameters used for QMRAspot and calculation of setback distances.

Pathogen	Parameter	Unit	Value	Reference
Cryptosporidium	River conc. (mean, 95th %)	n/l	0.15, 0.8	(Demeter et al., 2021)
	First order inactivation	day^{-1}	0.011	(Sidhu et al., 2010)
Campylobacter	River conc. (mean, 95th %)	n/l	0.8, 4.64	(Demeter et al., 2021)
	First order inactivation	day^{-1}	5 0.11	(Sidhu et al., 2010) (Ross and Donnison, 2006)

As a comparison to the model-derived 60-day TOT described above, the setback distance based on the Austrian 60-day TOT regulation was calculated by trial and error as per Eq. 7 (ÖVGW, 2004):

$$t = \frac{0.462 \bullet 0.045K_f}{U} \bullet \left[x - \frac{Q}{U \bullet h_{GW} \bullet 2\pi} \bullet ln\left(1+\frac{x \bullet U \bullet h_{GW} \bullet 2\pi}{Q}\right)\right] \quad (7)$$

where K_f is the hydraulic conductivity (L/T), U is the Darcy velocity (L/T) and h_{GW} is the height of the water column in the aquifer (L).

2.6. Upscaling of λ

In this study, the values for λ were found for a distance of 25 m and may not be representative for larger transport distances, as λ has been reported to decrease with an increase in travel distance (Hornstra et al., 2018; Kvitsand et al., 2015; Schijven and Šimůnek, 2002). Explanations for this phenomenon include distance-related straining processes, reduction of the ionic strength of the input solution over time due to dilution, blocking of favorable attachment sites, and bacterial heterogeneity (Bradford et al., 2007; Pang, 2009; Camesano et al., 1999). Because the necessary setback distances are likely an order of magnitude larger than this distance, the obtained values for λ from the experiments might yield setback distances that are too small, which could lead to potential health hazards. To solve this problem, an upscaled λ was estimated using the literature comparison in Oudega et al. (2021), from which a relationship between travel distance and λ can be formulated (Fig. 2). The relationship of y (corresponding to $\lambda_{field}/\lambda_{column}$) and x (corresponding to transport distance) was then used to calculate the ratio of $\lambda_{field}/\lambda_{column}$ at larger transport distances. Because the value of λ_{column} is known, λ_{field} can be calculated at a different transport distance. Because the relationship as found in Fig. 2 has no data points with a transport distance larger than 97 m (Harvey et al., 2008), scaling beyond this point would be unreliable. Therefore, a transport distance of 96 m was chosen as a characteristic length for upscaling λ.

Since this relationship is merely a first step to understanding the upscaling relationship at different transport distances, the resulting λ values have a very high uncertainty. Therefore, the upscaling of λ is used here only to showcase how setback distances could change if tracer tests are performed at different distances, highlighting the importance of reliably determining λ.

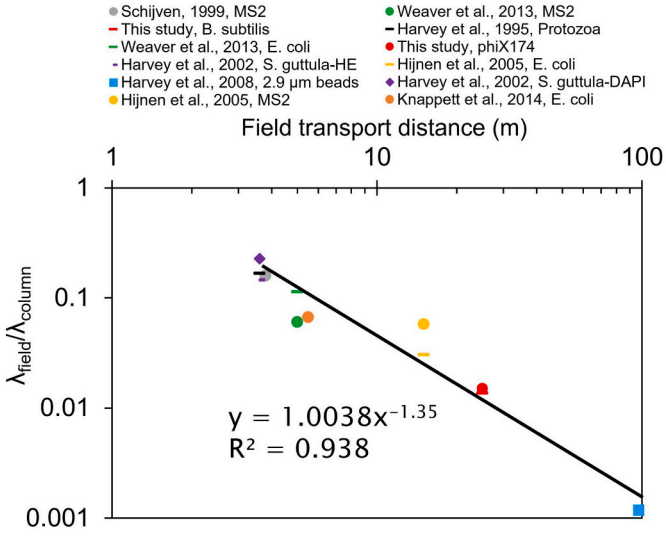

Fig. 2. Comparison of the ratio of $\lambda_{field}/\lambda_{column}$ between studies with different transport distances. Modified from Oudega et al. (2021).

3. Results

3.1. Comparison of bromide and uranine as conservative tracers

To ascertain that direct comparisons were possible between bromide and uranine, a test with both conservative tracers was carried out. The results show that bromide and uranine behave similarly in terms of peak timing and change in concentration (Fig. 3). Note that bromide was injected in a 100 times larger volume than uranine, hence the 2-log difference in C/C_0. This might also change flow properties (such as dispersion) between the tests, leading to slightly differing results. A further explanation of the differences between the two conservative tracers is that uranine might experience sorption to aquifer grains during subsurface flow (Dollinger et al., 2017). As bromide does not experience sorption, a higher concentration may be transported to the pumping well. Lastly, the difference in sampling and measurement methods might cause differences between the BTCs: bromide was sampled by an auto-sampler, while uranine was measured in the flow-through cell with a fluorescence meter, which is an advantage over sampling because of the high temporal resolution. The recovery rates were ~ 67.7% for bromide and ~ 63.3% for uranine. The similarities were considered sufficient to approve using uranine at this study site as a conservative tracer.

3.2. Bacillus subtilis spores breakthrough and model results

In tests 1a-b, the *B. subtilis* concentrations are in the range of the limit of quantification due to the low concentrations in the zed samples (Fig. 4-1a and 1b). At t ≈ 30 h, *B. subtilis* peaked earlier than the conservative tracer, showing that pore-size exclusion is an important process in the study area (Grindrod et al., 1996; Pang et al., 2005). This, in turn, affects the dispersivity values in the model, which were lower for *B. subtilis* than for the conservative tracers (Table 3). This is because the microbial tracers travel predominantly through larger and better-connected pores, leading to a more advective and less dispersive transport than the conservative tracers (Pang et al., 1998). The peak C/C_0 breakthrough was 4.2×10^{-9}, showing a peak reduction of just under 9-log. The very early breakthrough in test 1a was assumed to be contamination. Both tests exhibit significant tailing, as well as breakthrough of higher concentrations after the initial peak has passed (the high concentration in spore samples at $t = 60$ h in both tests 1a and 1b).

In tests 5a-b, peak concentrations were around 4 times higher than in tests 1a-b. Furthermore, peak time was much earlier than the conservative tracer peak. The reduction in peak concentration was approximately 8-logs. The outliers were likely a product of sample contamination.

In tests 10a-b, BTCs were less irregular than in the lower pumping rate tests, which can be attributed to the higher concentrations in the analyzed samples. Concentrations peaked at approximately $t = 4.69$ h with C/C_0 at 3.75×10^{-8}, around 3–4 times higher than tests 5a-b. An overview of the tests is shown in Table 3, with the removal rates for *B. subtilis* as calculated per Eq. 5, and values for K_{att} and K_{det} taken from the modeling study.

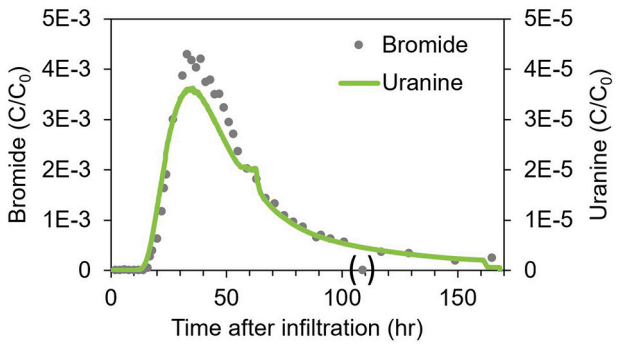

Fig. 3. Comparison of conservative tracers. Outliers between brackets.

4. Discussion

4.1. HYDRUS-3D modeling

The ratio of C_{max}/C_0 for *B. subtilis* spores in the tracer tests was generally 10^{-8}–10^{-9} compared to 10^{-3} for the conservative tracers (Table 3). This shows that removal processes are important for colloid transport in this area. Many studies have ascertained that no significant inactivation of *B. subtilis* spores took place after time periods from 7 days up to 45 days (Greskowiak et al., 2006; Li et al., 2018; Pang et al., 1998; Pike et al., 1969; Ratcliffe, 1995), and because the fraction of colloid size to median grain size (0.0003 in our experiments) is smaller than 0.0017, straining should not occur (Bradford et al., 2002). For these reasons, the removal of *B. subtilis* in this study was assumed to be governed by attachment.

Two attachment and detachments rate coefficients were necessary to accurately model the tailing of the *B. subtilis* BTCs. In this model, K_{att1} mainly controls the height of the peak concentration, while K_{att2}, K_{det1} and K_{det2} mainly control the shape of the falling limb of the BTC (Schijven et al., 2001). Table 3 shows the attachment and detachment rate coefficients that were found for each experiment. The difference between attachment sites can be explained by microbial population heterogeneity; i.e. certain *B. subtilis* spores within the colloidal population are inherently more or less likely to attach to sorption sites due to differences in their surface structure or charge (Foppen and Schijven, 2006; Schijven and Hassanizadeh, 2000). However, the more widely accepted explanation is that physical heterogeneity is more important; i.e. certain attachment sites are more likely to capture *B. subtilis* spores than others. This could depend on multiple factors, for example the extent of the favorable attachment sites, or their charge (i.e. iron content) (Ginn et al., 2002; Knapp et al., 1998; Schijven and Šimůnek, 2002).

It was found that K_{att1} was lowest for the lowest flow rate tests (1 l/s), but in the middle and highest flow rate tests (5 and 10 l/s respectively), K_{att1} was similar. Other studies have also found an increase in attachment rate with pore-water velocity (v) on both the column and field scale (Hendry et al., 1999; Hijnen et al., 2005; Schijven et al., 2001). K_{det1}, K_{att2} and K_{det2} were also found to increase with v. This shows that the ratio of reversibly attached particles increases with v, which affirms previous findings (Bradford et al., 2016b). More reversible attachment might be an important mechanism that causes removal to decrease with an increase in flow rate.

Even though the attachment rate coefficients increased with flow rate, overall removal decreased due to shorter residence times; a pumping rate change from 1 to 10 l/s decreased the removal rate (λ) by >30% (Table 3). This is supported by the literature on field tracer studies, which generally states that an increase in flow rate decreases removal (Camesano and Logan, 1998; Kvitsand et al., 2015; Pang, 2009), which in this study ranges from 0.19 to 0.28 log/m. Typically, values for λ are in the range of 10^{-2}–10^{-1} log/m for sand and gravel aquifers, but in gravel aquifers with a flow rate > 11 m/d, can be as low as 10^{-3} log/m (Pang, 2009). The values for λ in this study are on the higher side of this range, which might be due to the high amount of fine material present at this study's field site; the D_{50} is 4 mm versus, for example, 18 mm at the Burnham test site (Pang et al., 2005). Another explanation is that there could be differences in sphericity and/or surface roughness of the material, which can influence attachment (Saiers and Ryan, 2005; Shellenberger and Logan, 2002). Lastly, the high iron content in the groundwater of our study site points to the presence of iron-oxides, which can function as attachment sites, leading to enhanced microbial attachment (Ginn et al., 2002; Knapp et al., 1998).

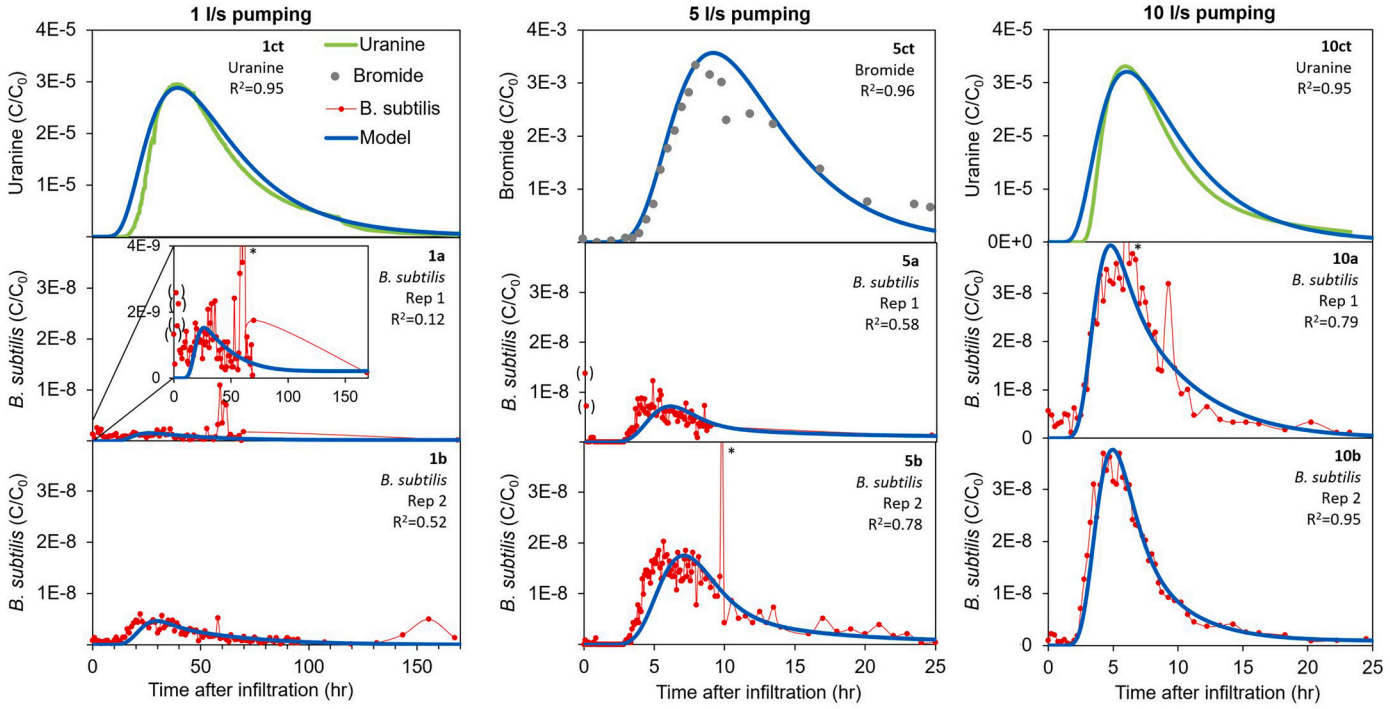

Fig. 4. The BTCs of the tracer tests and modeling study, where *ct* stands for conservative tracer test, and *a* and *b* are duplicates of the microbial tracer test. Asterisks (*) stand for outliers with concentrations above the maximum y-axis value. Figures *5ct*, *a* and *b* modified from Oudega et al. (2021).

Table 3
Results of field tests and modeling study.

	Test	1 Pre	1a	1b	5a[a]	5b[a]	10a	10b
	Pumping rate (l/s)	1.09	1.20	0.99	5.05	4.65	9.57	9.45
	Average flow rate (m/d)	2.44	2.36	1.90	14.75	13.36	24.36	23.45
	Gradient P24 – LB13 (‰)	7.51	5.30	5.85	13.16	12.61	24.66	25.34
Bromide	Peak concentration (C_{max}/C_0)	4.30×10^{-3}	–	–	3.34×10^{-3}	7.40×10^{-3}	–	–
	Recovery (%)	67.7	–	–	71.2	93.2	–	–
	Time to peak (h)	33.0	–	–	8.0	10.0	–	–
	Longitudinal dispersivity (m)	1.8	–	–	1.8	1.8	–	–
	Transverse dispersivity (m)	0.18	–	–	0.18	0.18	–	–
Uranine	Peak concentration (C_{max}/C_0)	3.62×10^{-5}	2.66×10^{-5}	2.93×10^{-5}	–	–	3.11×10^{-5}	2.87×10^{-5}
	Recovery (%)	63.3	58.1	51.3	–	–	74.8	84.0
	Time to peak (h)	35.5	48.0	39.2	–	–	5.8	6.1
	Longitudinal dispersivity (m)	2.9	2.9	2.9	–	–	2.9	2.9
	Transverse dispersivity (m)	1.1	1.1	1.1	–	–	1.1	1.1
B. subtilis	Peak concentration (C_{max}/C_0)	–	2.33×10^{-9}	5.96×10^{-9}	1.09×10^{-8}	2.03×10^{-8}	3.81×10^{-8}	3.69×10^{-8}
	Recovery (M_{out}/M_{in})	–	7.13×10^{-4}	6.93×10^{-4}	1.08×10^{-3}	1.75×10^{-3}	9.33×10^{-3}	7.47×10^{-3}
	Time to peak (h)	–	36.0	22.0	4.8	5.7	6.5	4.3
	Longitudinal dispersivity (m)	–	1.2	1.2	1.2	1.2	1.2	1.2
	Transverse dispersivity (m)	–	0.15	0.15	0.15	0.15	0.15	0.15
	λ (log/m)	–	0.284	0.274	0.273	0.257	0.189	0.198
	K_{att1} (hr^{-1})	–	0.18	0.12	0.76	0.60	0.53	0.66
	K_{det1} (hr^{-1})	–	0.0008	0	0.0040	0.0008	0.0001	0.0013
	K_{att2} (hr^{-1})	–	0.12	0.09	0.20	0.12	0.42	0.22
	K_{det2} (hr^{-1})	–	0.10	0.03	0.21	0.20	0.37	0.40

[a] Values taken from Oudega et al. (2021).

4.2. Setback distances

The computations with QMRAspot showed that to reach the health based target, the necessary total subsurface removal was 5.6 log for *Cryptosporidium* and 6.8 log for *Campylobacter*. The setback distances needed to realize this were calculated as per Eq. 6. In this equation, the values of λ found by the microbial tracer tests were used, as well as the values for the estimated upscaled-λ. As an additional comparison, a high and low value were used, taken from the range of λ for sand and gravel in the literature (Pang, 2009).

Table 4 shows setback distances calculated for each pumping rate, based on the 60-day TOT (as per Eq. 7, as well as extrapolated from model results), and based on the QMRA (Eq. 6) for each pathogen. The results show that a pumping rate increase from 1 to 10 l/s can lead to a 1.0–3.8 times greater setback distance, depending on the method used. Setback distances for *Campylobacter* were similar or smaller than for *Cryptosporidium* due to the higher inactivation rates (0.11–5 versus 0.011 log/day, respectively) (Sidhu et al., 2010). However, *Cryptosporidium* is larger than *B. subtilis* and might therefore experience additional removal processes such as straining and/or wedging (Headd and Bradford, 2016; Li et al., 2006; Tufenkji et al., 2004). Therefore, the setback distances for *Cryptosporidium* can be regarded as conservative. For low

Table 4
Calculated setback distances based on a 60-day TOT, as well as setback distances based on the QMRAspot for different values of λ.

Test	1a	1b	5a	5b	10a	10b
Experimental λ (log/m)	0.284	0.274	0.273[a]	0.257[a]	0.189	0.198
Estimated λ[b] (log/m)	0.028	0.027	0.027	0.025	0.018	0.019
Setback distances (m) based on 60-day TOT						
Model-derived[c]	477	523	585	524	736	657
Austrian regulation[d]	176	174	372	322	493	473
Setback distances (m) based on QMRAspot						
Cryptosporidium spp. (μ = 0.011 log/d)						
Using experimental λ	57	59	59	62	81	78
Using estimated λ	471	487	496	525	709	678
Using $\lambda = 10^{-1}$ log/m	142	142	143	143	143	143
Using $\lambda = 10^{-3}$ log/m	7752	7723	8153	8036	8394	8860
Campylobacter spp. (μ = 5–0.11 log/d)						
Using experimental λ	41–69	39–70	62–72	63–76	84–98	81–94
Using estimated λ	65–479	58–488	168–556	160–576	231–785	221–754
Using $\lambda = 10^{-1}$ log/m	55–164	50–163	109–171	106–170	124–172	123–172
Using $\lambda = 10^{-3}$ log/m	70–2146	62–2111	206–2819	190–2634	278–3176	266–3686

[a] Values taken from Oudega et al. (2021).
[b] The *estimated* (or upscaled) λ is the value acquired by the upscaling procedure based on Fig. 2, while the *experimental* λ is the value measured in this study.
[c] As calculated from the modeled groundwater gradient.
[d] As per Eq. 7.

values of λ, setback distances varied more with pumping rate than for higher λ. This is because at lower λ, inactivation becomes more important due to the longer travel times.

Compared to the 60-day TOT method, the setback distances as calculated by QMRA can be either smaller or larger, depending on the pathogen and the chosen value of λ (Table 4). The setback distances for both pathogens, as calculated with the estimated upscaled-λ (~700 m for the highest pumping rate), are similar to the model-derived 60-day TOT setback distances. This shows that, at least in this study, the best estimates for setback distances based on QMRA do not differ greatly from the 60-day TOT distance, and highlights that the TOT method is still viable if the necessary data for QMRA is not available, especially when considering its greater ease of use. However, whereas the 60-day TOT method is considered to be sufficient for all pathogens, the QMRA in this study was only done for *Cryptosporidium* and *Campylobacter*. Other pathogens might have different source concentrations, removal parameters and dose-response curves, which could lead to vastly different setback distances. Still, these results are in contrast with findings by Schijven et al. (2006), who calculated by QMRA that protection zones of 1–2 years were needed to reach the same health-based target. However, Schijven et al. (2006) did their analysis for viruses in raw sewage water leaking through a pipe into a uniform sand aquifer. The situation in this study is very different, as it assumes infiltration of river water through the riverbank. Even though pathogen concentrations in a river can increase drastically after heavy rainfalls, strongly affecting setback distances, this study accounts for this variability in source concentration by using results from a probabilistic-deterministic water quality model (Demeter et al., 2021).

Important parameters in the calculation of setback distances by QMRA are λ and inactivation rate (μ). Because it is not easy to find accurate values for these parameters, literature values are often used instead. A challenge is that a large range of values exists in the literature, even for the same aquifer type as is the case for λ in gravel aquifers (Pang, 2009). This is shown in Table 4, where using different λ for alluvial gravel aquifers can yield vastly different setback distances. In the case of *Campylobacter*, setback distances varied by 1–2 log, depending on which value of μ was chosen, which shows that the effect of μ on setback distance is greater than that of pumping rate (or flow gradient). This underlines the importance of obtaining reliable μ and λ values for the calculation of setback distances with QMRA. In-situ batch tests and field tracer tests are the most reliable methods to determine these parameters; however, besides the practical difficulties inherent in carrying out these tests, interpreting the resulting λ values poses further difficulties because λ can change with pumping rate as well as with scale. Therefore, it is additionally important to consider scaling for the calculation of setback distances with QMRA. Unfortunately, our methods of upscaling are not yet adequate for reliably estimating λ at larger scales. Ideally, there would be multiple sampling points to capture the effects of changing λ with distance, or microbial tracer tests would be done from the furthest possible point from the drinking water well, such as a river.

To be able to accurately calculate setback distances based on QMRA, it is important not only to obtain reliable transport parameters, but also to understand the hydrogeological setting. If the source of pathogens is a river, there might be additional inactivation due to sunlight exposure (Bambic et al., 2015; Rosa and Giuseppina., 2016). If the river has a static riverbank, a colmation layer might be present at the river-aquifer interface, which would dramatically increase removal (Derx et al., 2014; Derx et al., 2010). On the other hand, if the riverbank is natural, or if the likely source of pathogens is from a pipe located near the saturated zone, this attenuation effect would be largely diminished.

5. Conclusions

This study shows that when removal, λ, is low, varying the flow rate can have a large influence on setback distance calculations. Inactivation, μ, is also an extremely important parameter when the value of λ is small and setback distances are calculated with a QMRA approach. The literature range of λ (and in the case of some pathogens, μ) is very broad, which can lead to inaccurate setback distances. For this reason, tracer tests with surrogates should be performed at the site of interest, but care should be taken as to which surrogates are used, as well as which transport distance should be used to reliably determine λ. Unfortunately, it is not always possible to carry out tracer tests over large enough distances, and thus, it is important to create better and more reliable upscaling methods. To be able to do so, not only are more studies with microbial tracer tests needed at the column and field scale, but also more studies comparing different field scales (i.e. field studies in the same aquifer, but with different transport distances), as well as studies at the mesoscale, for example, in large (> 1 m) columns.

Funding

This work was supported by the Vienna Science and Technology Fund (WWTF) (Grant ESR17–070, "Giardy", and Grant ESR20–013, "Faterisk Aqua"), the Austrian Science Fund (FWF) as part of the DKplus (Vienna Doctoral Program on Water Resource Systems, W1219-N22) and the Austrian Academy of Sciences (Grant JF_2019_15, "Swim City"). M.E.S. received funding from the Austrian Science Fund (FWF)

(Grant T970-N29). Additional support came from the Vienna Water Resource Systems Project (ViWa 2020+), a research cooperation between Vienna Water (Municipal Department 31) and the ICC Water & Health.

CRediT authorship contribution statement

Thomas J. Oudega: Conceptualization, Methodology, Software, Writing – review & editing. **Gerhard Lindner:** Formal analysis, Methodology. **Regina Sommer:** Methodology. **Andreas H. Farnleitner:** Methodology. **Georg Kerber:** Methodology. **Julia Derx:** Conceptualization, Funding acquisition. **Margaret E. Stevenson:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing. **Alfred P. Blaschke:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing.

Declaration of Competing Interest

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

Julia Derx, Gerhard Lindner reports financial support was provided by Vienna Science and Technology Fund. A.P. Blaschke reports financial support was provided by Austrian Academy of Sciences. Margaret Stevenson reports financial support was provided by Austrian Science Fund. Thomas Oudega, Gerhard Lindner reports equipment, drugs, or supplies was provided by Vienna Water Resource Systems Project.

Acknowledgements

We thank Vienna Water for their permission, assistance and continued support during the tracer test studies.

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