

Characterizing aquifer heterogeneity using bacterial and bacteriophage tracers.

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Abstract:

Gravel aquifers act as important potable water sources in Central Western Europe, yet are subject to numerous contamination pressures. Compositional and textural heterogeneity makes protection zone delineation around groundwater supplies in these units challenging. Artificial tracer testing aids characterization. This paper re-appraises tracer test results, presented in Mallèn et al. (2005), in light of new geological and microbiological data. Comparative passive gradient testing, employing a fluorescent solute (Uranine), virus (H40/1 bacteriophage) and comparably-sized bacterial tracers *Escherichia coli* (*E.coli*) and *Pseudomonas putida* (*P.putida*), was used to investigate a calcareous gravel aquifer's ability to remove microbiological contaminants at a test site near Munich, Germany. Test results revealed *E.coli* relative recoveries could exceed those of H40/1 at monitoring wells, 10m and 20m from an injection well, by almost four times; *P.putida* recoveries varied by a factor of up to three between wells. Application of filtration theory suggested greater attenuation of H40/1, relative to similarly-charged *E.coli* occurred due to differences in microorganism size, while estimated collision efficiencies appeared comparable. By contrast, more positively charged *P.putida* experienced greater attenuation at one monitoring point, while lower attenuation rates at the second location indicated the influence of geochemical heterogeneity. Test findings proved consistent with observations from nearby fresh outcrops that suggested thin open framework gravel beds dominated mass transport in the aquifer, while discrete intervals containing stained clasts reflect localized geochemical heterogeneity. Study results highlight the utility of reconciling outcrop observations with artificial tracer test responses, using microbiological tracers with well-defined properties, to characterize aquifer heterogeneity. (250 words)

Nomenclature

| Symbol | Meaning (Units) | Symbol | Meaning (Units) |
|----------|--|-----------|---|
| k_c | First order rate deposition constant (d^{-1}) | γ | Collision efficiency (-) |
| C | Non-reactive tracer concentration ($\mu g.l^{-1}$) | η | Single collector efficiency (-) |
| v | Advective velocity ($m.d^{-1}$) | ρ_b | Bulk density (kg/m^3) |
| d | Collector (Grain) diameter (mm) | K | Hydraulic conductivity ($m.d^{-1}$) |
| θ | Porosity (-) | t | Time (d) |
| Tr | Reactive tracer concentration ($pfu.ml^{-1}$ or $cfu.ml^{-1}$) | μ_L | Inactivation rate in liquid (d^{-1}) |
| D_L | Longitudinal hydrodynamic dispersion ($m^2.d^{-1}$) | S | Concentration of attached particle (pfu/Kg or cfu/Kg) |
| RR | Relative recovery (-) | x | Distance (m) |
| μ_s | Inactivation rate on solid (d^{-1}) | k_{det} | Particle detachment rate (d^{-1}) |

Introduction

Unconfined sand and gravel (porous) aquifers encountered in areas surrounding mountainous regions of Central Western Europe act as important sources of potable water. In Switzerland, these units provide approximately 36% of all drinking water (BAFU, 2015), while surrounding states display similar levels of reliance (EEA, 1999). Immediately to the north of Switzerland groundwater, largely derived from comparable porous aquifers, accounts for approximately 90% of water supplies in Bavaria (Germany) (BGR, 2010). Despite their economic importance, intense agricultural and industrial land use across the region places porous aquifers at elevated risk of impacts from chemical and microbiological contaminants, including pathogenic microorganisms. These pressures make preventing or limiting inputs of pollutants to groundwater, as required under the EU water Groundwater Directive (2006/118/EC) and Water Framework Directive (2000/60/EC), a challenging task.

Source protection schemes aim to identify areas around groundwater sources where land use restrictions may be applied to reduce risk of contaminants entering water supplies (Carey et al., 2009). Definition of these areas requires an understanding of aquifer characteristics, particularly for pathogenic microorganisms, where travel times between sources and receptors can influence concentrations that remain viable (and capable of infection). Although travel times through porous aquifers are considered quite slow and relatively uniform (BAFU, 2015) high levels of textural heterogeneity have been noted in these units over short distances (Huggenberger and Regli, 2006), suggesting that hydraulic conductivity can prove highly variable. This, in turn, can give rise to spatial variations in groundwater flow rates.

Artificial tracer testing permits characterization of mass transport processes in aquifers (Käss, 1998). Apart from qualitatively demonstrating hydraulic connection between injection and observation points (, surrogates for sources and receptors), tracer concentration history at an observation point may be represented as a breakthrough curve (BTC), whose analysis can provide an indication of the transport characteristics of aquifer. These analyses, in turn, provide a means of determining a groundwater system's ability to dilute contaminants, verify

groundwater protection zones and quantify mass transport processes (Schaefer et al., 2004; Auckenthaler et al., 2002; Schijven et al., 1999; Hinsby et al., 1996).

Where quantification of reactive contaminant attenuation rates is required, comparative tracer testing, in which the relative responses of reactive and non-reactive tracers are compared, provides a means of constraining mechanisms and rates of contaminant attenuation. This approach has been applied in hydrogeological research to investigate the transport and attenuation of colloidal tracers, including microorganisms such as bacteria and viruses (e.g. Harvey and Garabedian, 1993, Schijven et al., 2000). Under these circumstances, reactive tracers are typically assumed to have the same transport characteristics as non-reactive tracers, with any differences in response attributed to reaction.

Quantification of reactive contaminant attenuation processes, employing mathematical models, provides a basis for comparing and predicting contaminant responses while accounting for differences in hydrodynamics and intrinsic aquifer properties. BTC modelling proves particularly challenging in natural porous media, including heterogeneous sand and gravel deposits, where conditions often depart from idealized homogeneous assumptions. Conceptual models of aquifer structure provide a means of linking tracer responses to realistic geological frameworks.

Data used in conceptual model development are often based on borehole samples. However, the quality of these samples may prove highly variable depending on sampling methodology, particularly when collected using older drilling methods, such as percussion drilling, which can provide disturbed composites of different geological units. This can lead to a loss in resolution and failure to adequately characterize deposits from thinner units. Anderson et al. (1999) recognized the value of datasets derived from outcrop exposures for providing supplemental high-resolution data about aquifer geometry (outcrop analogies). Huggenberger and Aigner (1999) adopted this approach to refine existing geological models of Swiss sand and gravel aquifers and generate a more confident conceptual basis for developing wellhead protection zones.

Although aquifer heterogeneity complicates understanding of fate and transport, knowledge of fundamental processes influencing contaminant mobility is necessary to develop a scientifically-defensible basis for deepening understanding of reactive tracer responses and encourage their wider application. Measurements completed under controlled laboratory conditions can allow characterization of the processes that influence reactive tracer behavior and tracer responses, observed in the field, to be reconciled with physical and chemical fundamentals. Filtration theory, in particular, provides a basis for distinguishing the relative importance of physical and chemical processes affecting the fate and transport of particles in saturated porous media (Kretzschmar et al., 1999). Consideration of microorganisms as suspended particles, particularly in fast flowing systems, has allowed application of filtration theory to investigate their fate and transport (Flynn et al., 2004a; Sinton et al., 2010). However, consensus about the best means of applying the theory remains to be resolved (Bradford et al., 2014). Tufenkji (2007) noted that many outstanding issues relate to reconciling chemical and electrostatic aspects of particle-surface interaction at the particle-grain scale. Conversely, more recent versions of the theory provide a more thorough basis for understanding the influence of physical variables (Tufenkji and Elimelech, 2004). Shortcomings of chemical and electrostatic elements of the filtration theory can be addressed at larger scales by employing empirical collision efficiencies based on tracer breakthrough curve responses (Kretzschmar et al. 1997). Other outstanding issues may either prove

irrelevant for particular microbiological tracer-groundwater-aquifer systems, or may be explicitly incorporated into model expressions.

Filtration theory permits attenuation rates to be explained in terms of physical and chemical interactions between collector surfaces (aquifer grains) and particles (microorganisms) while accounting for system hydrodynamics, i.e. the method permits hydrodynamic influences on attenuation rates to be factored out to better characterize particle-collector surface interactions. As a corollary to this point, responses of microorganisms may be explained in terms of interactions with media through which they pass, where contrasting fixed (aquifer) surfaces can generate different attenuation rates. Consequently, introduction of microbiological tracers, having well-characterized properties, into an aquifer has the potential to permit subsurface conditions, including aquifer heterogeneity, to be better defined.

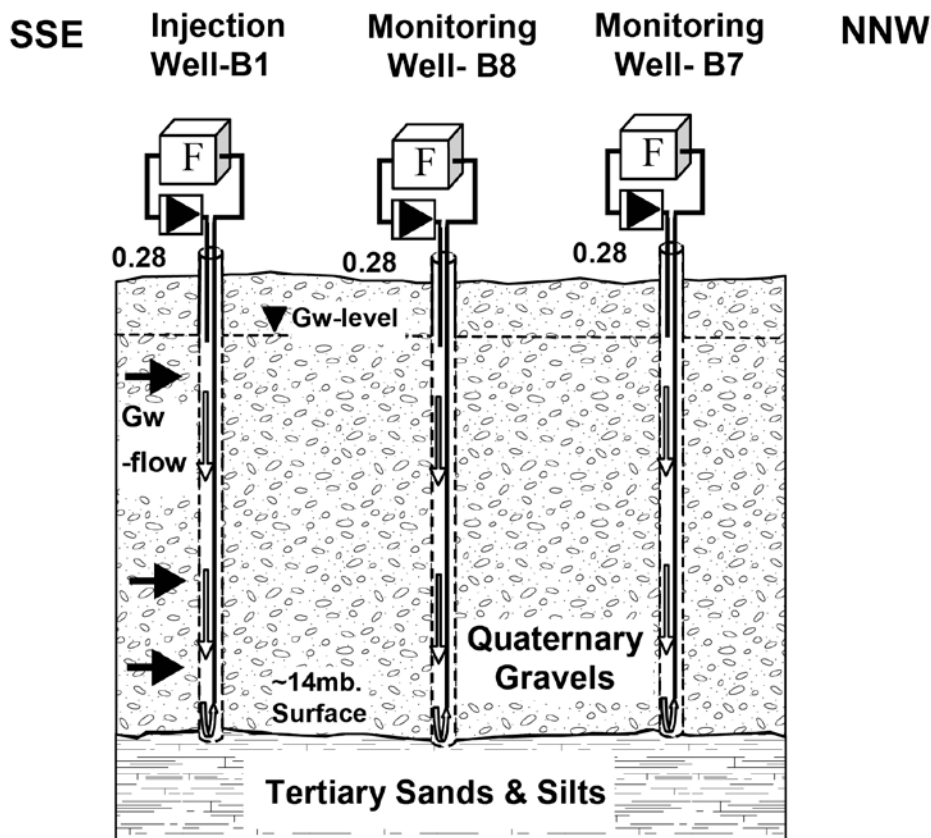
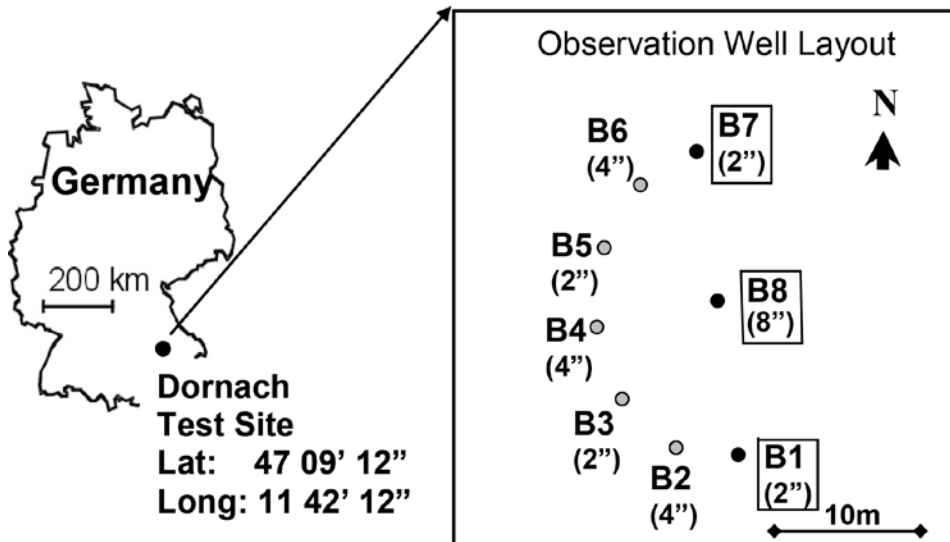
This paper examines the responses of three differently-sized microorganisms (two types of bacteria and a bacteriophage virus) employed in passive gradient comparative tracer tests, carried out in heterogeneous calcareous sand and gravel aquifer in southern Germany. Use of comparably-sized microbiological tracers with different surface characteristics, and differently-sized microorganisms with comparable surface properties, provided a means of investigating the influence of subsurface aquifer heterogeneities and reconciling microbiological tracer responses with geological conditions observed in nearby sand and gravel exposures.

Materials and Methods

Test Site Description

The Dornach Test Site, near Munich, Southern Germany (Dornach) is underlain by a 12m-14m sequence of Quaternary sand and gravel deposits overlying Tertiary sands and silts (Figure 1). The sand and gravel aquifer forms part of a more extensive groundwater body, with areas to the south being dominated narrower north-south trending units, laterally isolated from one another. The aquifers form part of a continuum with more localized deposits that also occur in Switzerland and to the east in Austria, where they also act as important drinking water sources. Artinger et al. (1996) reported that average flow velocities in these gravel units can reach 15m/day to 45m/day, while depths to groundwater in the south are typically around 13m below ground surface (mBGS) in the south rising to 2mBGS further north. Geochemically many of these units are dominated by carbonate and contrast with siliciclastic sands and gravels more widely encountered on the southern side of the Alps.

In the vicinity of Dornach, intensively farmed agricultural land surrounds the site, with subordinate areas devoted to gravel quarrying and light industrial activity/settlement also encountered within 1km. Mallèn et al. (2005) provided a detailed description of the site's tracer monitoring instrumentation. Briefly, Dornach consists of eight percussion-drilled fully-penetrating wells, screened across the entire saturated thickness of the underlying gravel aquifer (Figure 1). Grain size analyses of borehole samples revealed a wide variation in textures, ranging from fine sands to sand and gravel sequences; these occasionally contain localized iron-oxide stained beds. Zahn and Seiler (1992) estimated the gravels to have an effective porosity of 11%. Hydrochemical monitoring of site groundwater revealed it to have a relatively consistent, near-neutral pH and an ionic strength dominated by calcium bicarbonate (Seiler and Müller, 2001). Hydraulic gradients measured across the site were very gentle (<0.001)



F- On-line Fluorometer

▣ -Circulation Pump

Figure 1: Location map of the Dornach Test Site, Germany with schematic showing details of underlying geology and groundwater monitoring network. Monitoring points denoted by solid black dots were those employed in the comparative tracer tests described. (Modified from Müller & Seiler, 2001).

Artificial tracer testing, completed prior to the current phase of investigation, revealed that fluorescent solute and ionic tracers flowed from the injection well, B1, to observation wells,

B7 and B8, to the north northwest with average groundwater flow rates in excess of 10 m/day (Seiler & Muller, 2001). Further testing by Flynn et al. (2005), using a mobile downhole fluorimeter to monitor depth-specific fluorescent solute tracer arrival in the 200mm (8 inch) observation well(B8) and revealed that virtually all (>95%) Uranine tracer injected arrived in discrete intervals no thicker than 50cm.

Outcrop Analogy Study

In order to better evaluate the heterogeneity of Munich Gravel Plain Aquifer deposits in the vicinity of Dornach, sand and gravel sequences from the uppermost three meters of the unit were examined at gravel pits within 1 km of the site during a period of low groundwater levels. Fresh exposures, up to 3m deep by 4m long, permitted the dimensions and composition of sedimentary architectural elements to be examined in detail and samples of each unit to be collected for grain size distribution analysis using the method employed by Flynn et al. (2004a). Application of 1M HCl to samples confirmed the dominance of calcium carbonate (calcite), with subordinate quantities of dolomite, as the principal material making up the aquifer. These findings are consistent with those reported by Artinger et al. (1996) for gravel samples collected in the same area. Subsequent grain size analyses of samples, by wet sieving, permitted determination of the relative proportions of grain size fractions greater than 63µm in diameter.

Microbiological Tracer Surface Property Characterization.

Microbiological tracer surface property characterization consisted of measurements of zeta potential and of contact angles to provide an indication of contrasts in surface charge and hydrophobicity of each tracer employed. Bacterial sample preparation involved the *E.coli* strain K12 (DSMZ 498) and *P.putida* strain F1 (DSMZ 6899), used in the tracer tests at Dornach, being grown overnight in 500 ml sterile glass containers with 250 ml of their respective culture medium, placed on a rotating shaker. *E.coli* was grown in LB medium (pH 7.5) at 37°C. *P putida* was grown in CASO bouillon (DSMZ 220) at 28°C. Bacterial cells were harvested by centrifugation (5000 x g, 15 minutes) and the pellet was washed twice with artificial calcareous groundwater (AGW), prepared following the approach described in Flynn et al. (2004b), to remove both exopolymeric substances and soluble proteins from the culture medium. Finally the pellet was re-suspended in 50 ml of AGW. Cells were used within one hour in the subsequent analysis.

A suspension of the marine bacteriophage, H40/1, was prepared following the approach described in Rossi (1994). The suspension was centrifuged at 10000xg for 20 minutes to remove bacterial debris. Chloroform was added to the supernatant (1:1 proportion) and the suspension was shaken for five minutes to precipitate dissolved bacterial and culture medium components. After centrifugation (5000 x g, 10 min), the H40/1 supernatant was collected and filtered through a 0.22 µm cellulose ester filter (Millipore, Schaffhausen, Switzerland). Bacteriophage were precipitated using polyethylene glycol as recommended by Yamamoto et al. (1970). The pellet was re-suspended in 10 mM NaCl (buffered to pH 7.4).

Zeta potential Measurements: Zeta potential was measured on a ZetaSizer Nano-ZS (Malvern Instruments Ltd., Malvern, UK) according to the manufacturer instructions. A 50 µl cell suspension (ca. 10⁶ cells) was mixed with 1 ml of AGW (pH 7.6, Electric conductivity 600 uS/cm) and run at 150 mV in disposable measuring cells (Malvern Instruments Ltd., Malvern, UK). The same approach was adopted for H40/1 bacteriophage, except that the viral suspension (ca 5x10¹¹ particles) was re-suspended in 10mM PBS buffer (pH 7.4) after purification.

Contact Angle Measurements: A layer of either *E.coli* or *P. Putida* cells was deposited by filtration onto a 0.2 µm sterile 48 mm diameter nitrate cellulose filters (Sartorius, Epsom, UK) until the almost complete saturation of the filters was achieved. The cell layer was washed with 25 ml of AGW and air-dried for 1 hour before being sliced and glued onto glass slides using double-sided film (Pritt, Cheshire, UK).

H40/1: A suspension of bacteriophage containing 2×10^{10} to 1×10^{11} viral particles was filtered through a cellulose acetate filter (0.025 µm, diam 2,5 cm) (Millipore, Schaffhausen, Switzerland). The filter was fixed with double-sided tape on a glass slide and air dried for at least 1 hour at room temperature.

Contact angle measurements were carried out on a G10 Contact Angle Measuring System (Krüss, Hamburg, Germany). Visual observations were made with 10 µl drops of each microbiological tracer suspended in AGW. Results were averaged from at least ten measurements. Measurements controls were composed of clean glass and Parafilm (Sigma-Aldrich, Andover, UK) surfaces.

Tracer Test Methodology

A program of artificial tracer tests aimed to investigate solute, bacterial and viral tracer relative responses at observation wells B7 and B8, following injection at B1, with a view to reconciling attenuation characteristics with aquifer heterogeneity. Table 1 summarizes salient details of the tracers employed. Two tracer tests, completed on successive days, investigated Uranine, *Escherichia coli* DSM 498 (*E.coli*) and H40/1 bacteriophage (H40/1) transport and attenuation. Two further tests, completed on successive days, at roughly the same time in the following year, investigated Uranine transport compared to *Pseudomonas putida* (*P.putida*) and H40/1.

| Tracer | Type | Mass Injected (Date Injected) |
|-----------------|---------------------------------------|--|
| Uranine | Solute | 100 mg (July 2000) 102 mg (May 2001) |
| <i>P.putida</i> | Bacterium | 4×10^{10} cfu (May 2001) |
| <i>E.coli</i> | Bacterium | 2×10^{10} cfu (July 2000) |
| H40/1 | Bacteriophage (Bacterial virus) | 2.3×10^{13} pfu (July 2000) 1.32×10^{12} pfu (may 2001) |

Table 1: Summary of details of tracers employed at Dornach Test Site for tests completed in July 2000 and May 2001.

As noted, the experimental program aimed to evaluate the utility of microbiological tracers, when combined with filtration theory, to investigate heterogeneous porous aquifers. This involved comparing responses of similarly-sized microbes with contrasting surface properties (*P.putida* and *E.coli*) and differently-sized virus and bacterial tracers with similar surface charge characteristics (*E.coli* and H40/1). Use of the smaller H40/1 bacteriophage tracer in both series of investigations provided a common baseline by which the responses of the two bacterial tracers could be compared.

According to filtration theory, smaller particles with comparable surface properties should experience greater degrees of attenuation, while comparably-sized particles with more negative surface charge (, as reflected by more negative zeta potential,) should experience

less attenuation when passing through a medium dominated by negatively-charged surfaces (unfavorable deposition); high recoveries of H40/1 in experiments completed by Flynn et al. (2004b), using calcite sands and calcareous synthetic freshwater, under comparable conditions to those encountered at Dornach, indicated that calcite is repulsive to H40/1 near neutral pH.

Tracer injection into an active recirculation system, which pumped groundwater from the base of B1 to the ground surface, before discharging to the water table, allowed a slug of tracer cocktail (Uranine and microbiological tracer) to pass across the entire saturated thickness of the aquifer. This system permitted sampling and monitoring without disturbing the ambient (passive) hydraulic gradient across the site. On-line fluorimeters measured Uranine concentrations at ground surface and showed that the tracer's concentration rapidly dropped below the detection levels within 3 minutes following injection.

Identical recirculation systems monitored Uranine levels in groundwater at monitoring wells B7 and B8. Separate grab samples, collected at 15 minute to one hour intervals from the monitoring wells, permitted groundwater analyses for microbiological tracers to be completed; this was complemented by lab-based analysis of Uranine to verify that on-line instrumentation operated properly. Immediate refrigeration of grab samples ensured that the possibility of microorganism inactivation (loss of infective capacity) due to excessive temperatures at the ground surface was minimized. All laboratory-based microbiological analyses were initiated within 24 hours of sample collection.

Mallèn et al. (2005) provide details of culturing methodologies used for determining the concentrations of microbiological tracers in groundwater samples collected; the authors also reported that inactivation rates (loss of infective capacity) of suspended microorganisms, determined in the laboratory using formation water, at temperatures comparable to those in the aquifer, were negligible over the time frame of the tracer tests (two days).

Data Analysis

The advective dispersive equation (ADE) provides a basis for simulating BTCs generated in saturated porous media. For the tests completed at Dornach, comparison of simulation outputs using two-dimensional and three-dimensional solutions with the one-dimensional form of the ADE, carried out by Mallèn et al (2003), demonstrated that responses observed at B7 and B8 can be approximated using a one-dimensional solution for non-reactive tracers, as follows:

$$\frac{\partial C}{\partial t} = D_L \frac{\partial^2 C}{\partial x^2} - v \frac{\partial C}{\partial x} \quad (1)$$

, with tracer breakthrough simulated by variations in advective velocity and dispersion

Schijven et al.(1999) modified this approach to account for microorganism (virus) removal.

$$\frac{\partial Tr}{\partial t} = D_L \frac{\partial^2 Tr}{\partial x^2} - v \frac{\partial Tr}{\partial x} - k_c Tr - \mu_l C + k_{det} \frac{\rho_b}{\theta} S \quad (2)$$

$$\frac{\rho_b}{\theta} \frac{\partial S}{\partial t} = k_c - k_{det} \frac{\rho_b}{\theta} - \mu_s \frac{\rho_b}{\theta} S \quad (3)$$

Sinton et al. (2010) employed a comparable approach to model bacterial responses in coarse-grained porous media. Equation (2) and Equation (3) incorporate attenuation by first-order kinetic adsorption, desorption and inactivation. Schijven et al. (2000) demonstrated that

inactivation of deposited phases may be determined from residual tracer tailing following the passage of the peak and recession limb of the main body of the breakthrough curve. Since the independent experimental measurements made by Mallèn et al. (2005) revealed that inactivation rates in suspension of all microbiological tracers employed was negligible over the time frame of the tests, this permitted modification of Equation (2) to the following :

$$\frac{\partial Tr}{\partial t} = D_L \frac{\partial^2 Tr}{\partial x^2} - v \frac{\partial Tr}{\partial x} - k_c Tr + k_{det} \frac{\rho_b}{\theta} S \quad (4)$$

The number of variables employed in Equation (4) makes fitting observed field data BTC responses to the ADE challenging. Independently derived variables can provide further control. Comparison of responses of cumulative breakthrough curves for reactive and non-reactive tracers can be expressed by relative recovery (RR) (Harvey and Garabedian, 1992):

$$RR = \frac{\int_0^T Tr dt}{\int_0^T C dt} \quad (5)$$

Relative recovery was utilized with hydrodynamic parameters to assist in quantifying mass transport of reactive materials and the degree of reactive attenuation experienced. The model code virus.exe (Cornaton, F., presented in Flynn, 2003) was used to iteratively generate tracer best fit BTC responses. For the purposes of simulations, modelling assumed linear flow paths between injection and observation points. Adjustment of transport and reactive attenuation terms to fit microbiological tracer relative recovery and BTCs allowed simulations to quantify model variables, including deposition constants.

Investigations into the influences controlling the value of the deposition constant, k_c , using colloid filtration theory, demonstrated that its value may be related to the properties of the filter medium and particle as follows (Yao et al., 1971):

$$k_c = \frac{3(1-\theta)}{2d} \gamma \eta v \quad (6)$$

, where the value of γ (collision efficiency), expressing the probability of colliding particles sticking to fixed surfaces, is dominated by the chemical properties of the particle-fixed surface-solution system (Kretzschmar et al., 1999). By contrast values of η , reflecting the frequency of particle collision with fixed surfaces, are more influenced by the physical properties of the system, including the dimensions of suspended particles and fixed collectors (Kretzschmar et al., 1999).

Tufenkji and Elimelech (2004) presented details of an approach for calculating η , determined by summing contributions from Brownian diffusion, gravitational sedimentation and interception. The value of η reflects the interrelationship between flow dynamics, collector (aquifer grain) size and particle (microbiological tracer) diameter. Kretzschmar et al. (1999) noted that heterogeneous conditions encountered in natural deposits complicate understanding particle (, including microorganism,) transport and attenuation. For the purposes of field investigations levels of physical and compositional aquifer heterogeneity cannot be quantified at high spatial resolution. To address this issue global equivalent collision efficiency values, based on geological observations, were adopted. Dealing with the wide variation in possible collector size proved particularly challenging. Following the approach of Martin et al.(1996) the d_{10} (size of the finest 10%) from grain size distribution

curves was considered most appropriate. The d_{10} values, along with a particle density of 1050 kg/m^3 , a Hamaker constant of $3 \times 10^{-20} \text{ J}$ and an ambient water temperature of 10 Deg C permitted calculation of collector efficiency for a given flow velocity. Use of these parameters in Equation (6) permitted estimation of collision efficiencies using deposition constants, determined through ADE modelling, for each microbiological tracer BTC. The approach, although not exhaustive, provides a means of assessing the relative influences of physical and chemical aspects of the microorganism-groundwater-particle system on microbiological tracer response. Adopting this approach for the range of microbiological tracers employed permitted relative values of collision efficiency to be determined and compared across the suite of tracer tests completed. Findings were then compared with microbiological tracer surface properties to examine the relative influences of chemical and physical processes on attenuation rates.

Results

Outcrop Analogy Studies

Fresh exposures of Munich Gravel Plain sands and gravels at quarries in the immediate vicinity of the Dornach Test Site displayed high levels of heterogeneity in the deposits forming the upper layers of the formation. Observations at all sites revealed up to one meter of silty gravel overlying beds of sandy gravels that can exceed one meter in thickness. These in turn enclosed lenses of 10cm-50cm thick clean open-framework (silt free) gravels (OWG), which could extend laterally for over four meters. Due to difficulties associated with accessibility, only one exposure could be sampled for grain size analyses. Nonetheless, visual investigation of exposures across the area showed comparable OWG deposits to occur at a number of locations in the area.

Figure 2 summarizes the results of granulometric analyses for representative samples of the deposits observed. The data provide an indication of the degree of hydraulic conductivity heterogeneity that may be anticipated at the meter scale. It is apparent from the analyses that d_{10} ranges from silt-sized diameters (<63 microns) in the silty gravels, encountered just below the ground, to granule-sized ($>1\text{mm}$) for the OW gravels. Corresponding hydraulic conductivities estimated for these deposits using the Hazen Rule (1911) range from approximately 2000 m/day for OWG to less than 0.1 m/day for the silty gravels; values calculated for the sandy gravels range between 1 and 25 m/day . Observations of most OWG beds revealed them to be dominated by unstained calcium carbonate, localized black staining was observed covering grains (clasts) in particular beds, while red-brown staining was also apparent elsewhere. Similar staining has been recorded in samples collected from below the test site (Seiler and Müller, 2001), where iron oxide staining was noted in samples collected from a number of boreholes.

Microbiological Tracer Characterization

Table 2 summarizes the results of zeta potential and contact angle measurements for the microbiological tracers employed. Results show that under hydrochemical conditions, comparable to those encountered in groundwater below the Dornach Test Site, both *E.coli* and H40/1 have comparable zeta potentials, indicating similar gross surface charge characteristics. Values for *P.putida* proved more positive. Conversely, while contact angle measurements suggested that all tracers were hydrophilic, results for *P.putida* suggested that it was considerably more so than either H40/1 or *E.coli*. (13.5 Degrees for *P.putida* compared to 23.6 Degrees for *E.coli* and 52 Degrees for H40/1)

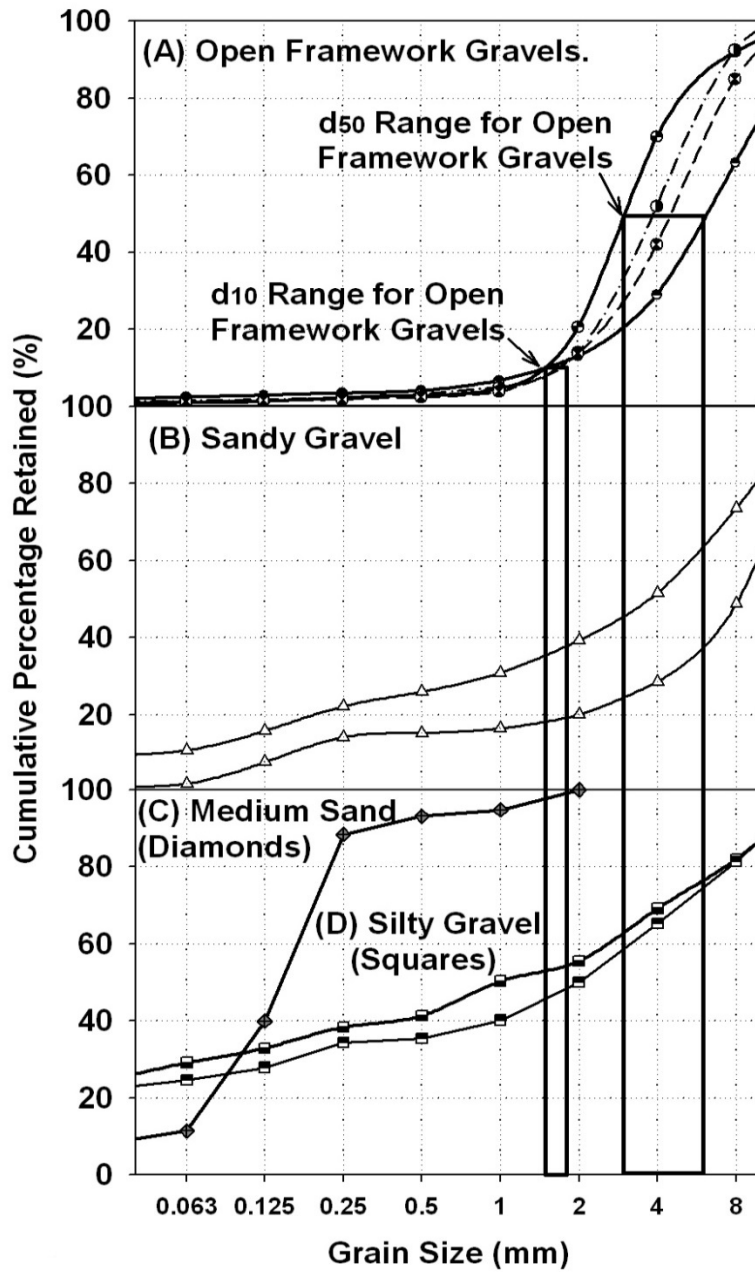


Figure 2: Grain size distribution curves for samples collected from gravel pits within 1km of the Dornach Test Site. Sandy gravel and silty gravel sample grain size ranges compare favorably with ranges measured in samples collected from boreholes at the test site.

| Microbiological Tracer (and dimensions) | Zeta potential (mV) | Contact angle (degrees) |
|--|----------------------|----------------------------|
| <i>Pseudomonas putida</i> (1.0 μm x 3-4 μm) | -17 \pm 0.12 (n=3) | 13.5 (n=10) |
| <i>Escherichia coli</i> (0.5 μm x 2.0 μm) | -25 \pm 0.15 (n=3) | 23.6 (n=10) |
| H40/1 (40nm x 80nm) | -23 \pm 1 (n=5) | 52 (n=6) |

Table 2: Summary table of microbiological tracer surface property characterization results.

Tracer test analyses

Figure 3 and Figure 4 present the results of BTCs generated for tracer tests completed at B7 and B8 respectively, along with modelled microbiological tracer responses. Comparison of Uranine curves for tests completed on successive days reveal little contrast in response and suggest that levels of the solute tracer return to background within 48 hours of injection. On the other hand, solute BTCs differ significantly from those generated using microbiological tracers, with modelled transport parameters derived from solute tracer responses providing unsatisfactory results to permit microbiological tracer BTCs to be simulated. As a consequence, modelling efforts focused on reproducing microbiological tracer breakthrough curves by adjusting values of v and D , while using relative recovery to estimate reactive attenuation parameters.

| | Advective Velocity ² (m.d ⁻¹) | Deposition Constant (day ⁻¹) | Dispersion (m ² .day ⁻¹) | Release Constant (day ⁻¹) | Inactivation rate on solid (d ⁻¹) | Estimated Collision Efficiency ³ | Relative Recovery (%) |
|-----------|--|--|---|---------------------------------------|---|---|-----------------------|
| B7 | | | | | | | |
| P.putida | 87 | 3.53 | 37.49 | 0.19 | 2.16 | 1.77E-03 | 52 |
| H40/1 | | 6.31 | 38.04 | n/c ¹ | n/c ¹ | 3.83E-03 | 28 |
| E.coli | 82 | 4.22 | 36.29 | 0.36 | 0.01 | 4.55E-03 | 63(76) ⁴ |
| H40/1 | | 8.83 | 34.34 | 0.1 | 1.44 | 5.45E-03 | 17 |
| B8 | | | | | | | |
| P.putida | 45.5 | 9.26 | 4.32 | 0.19 | 2.4 | 7.88E-03 | 17 |
| H40/1 | | 8.62 | 7.39 | n/c ¹ | n/c ¹ | 6.00E-03 | 20 |
| E.coli | 46 | 2.9 | 8.4 | 0.48 | 0.01 | 4.80E-03 | 77(90) ⁴ |
| H40/1 | | 6.6 | 7.75 | 0.19 | 2.4 | 4.81E-03 | 32 |

Table 3: Summary of model input parameters used to fit microbiological tracer breakthrough curves for B7 and B8 observation wells at the Dornach Test Site, Munich, Germany.

Notes: 1. Could not be determined from breakthrough curve due to injection of second tracer pulse. However, sensitivity analysis indicated that omission of these terms for H40/1 gave an increase deposition in constant of less than 10%.

2. Velocities estimated based on assumed linear trajectories between injection and observation wells.

3. Collision efficiencies estimated based on assumed 1.5mm diameter collector, 11% porosity, 10 Degrees C water temperature, particle density of 1050 kg.m⁻³ and a Hamaker constant of 3x10⁻²⁰ J.

4. Relative recovery calculated 24 hours after injection. Value in parenthesis is relative recovery 48 hours after injection. Where incomplete solute tracer breakthrough was observed, trends on the recession limb of Uranine BTCs were extrapolated, using a log-linear best fit line, to background. In the experiments described this generated no more than an additional 5% of total Uranine recovery.

Table 3 presents relative recoveries for each microbiological tracer, compared to Uranine, along with estimated deposition constants, determined using Equation (3) and Equation (4). Test results reveal marked contrasts between the responses of the three microbiological tracers. The table also presents estimated collision efficiencies, calculated from deposition constants using Equation (6). The values display little variation between wells in tests employing the *E.coli*/H40/1 microbiological tracer pair for each microorganism. Moreover, similar values calculated for the two microorganisms (4.6x10⁻³ to 4.8 x10⁻³ for *E.coli* vs

4.6×10^{-3} to 5.5×10^{-3} for H40/1) comparable favorably with their zeta potentials (-25mV for *E.coli* vs -23mV for H40/1).

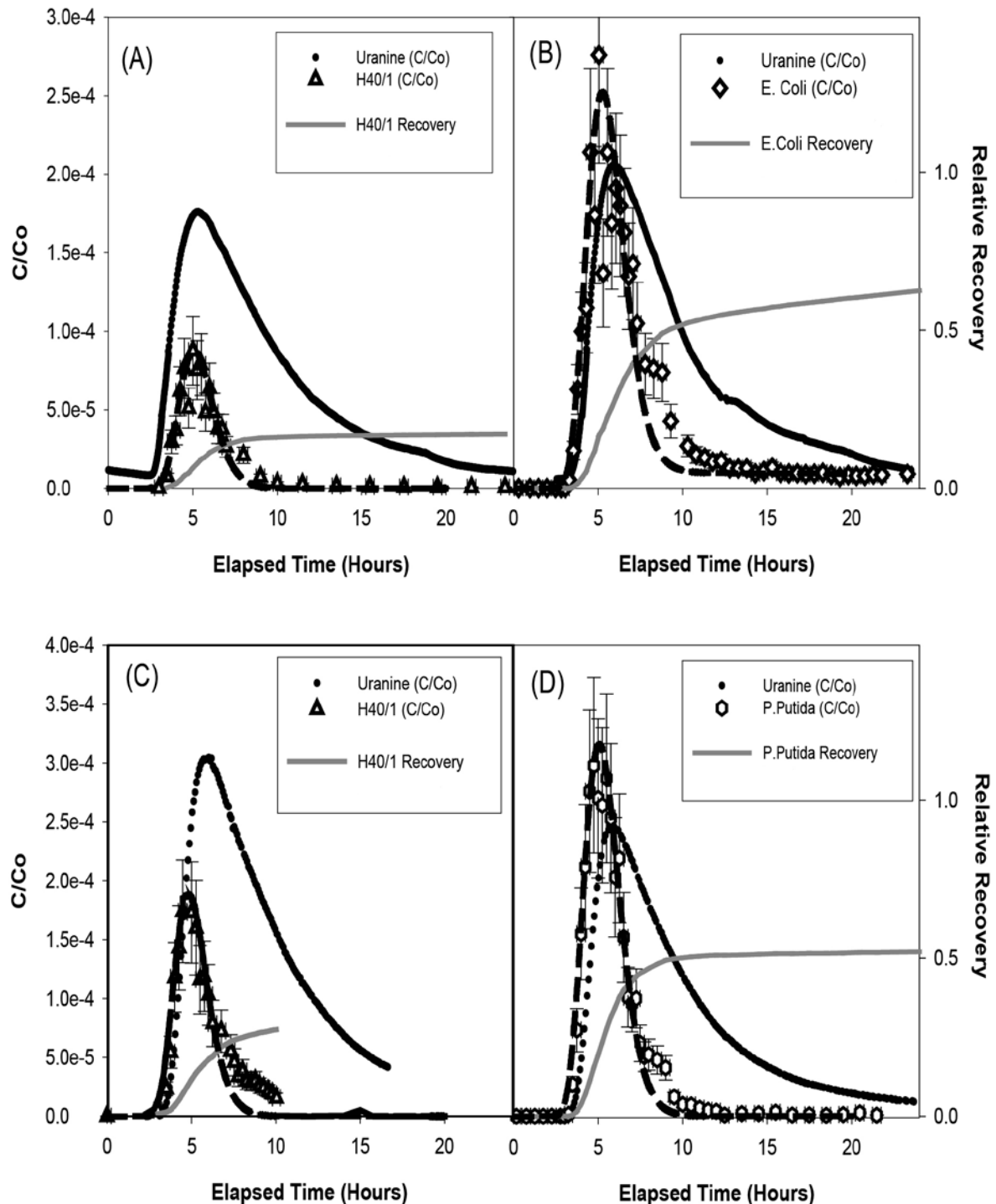


Figure 3: Solute and microbiological tracer breakthrough curves for monitoring well B7. Solute breakthrough curves digitized from hardcopy print-out. (A) *H40/1* & Uranine, 18 July 2000. (B) *E.coli* & Uranine, 17 July 2000. (*E. coli* Background Conc.: 2.25×10^{-8}). (C) *H40/1* & Uranine, 16 May 2001. (D) *P.putida* & Uranine, 15 July 2000. Modeled microbiological tracer breakthrough shown as black dashed lines. Temporal sampling error ± 0.25 hours. Analytical microbiological tracer error $\pm 25\%$ of measured conc.

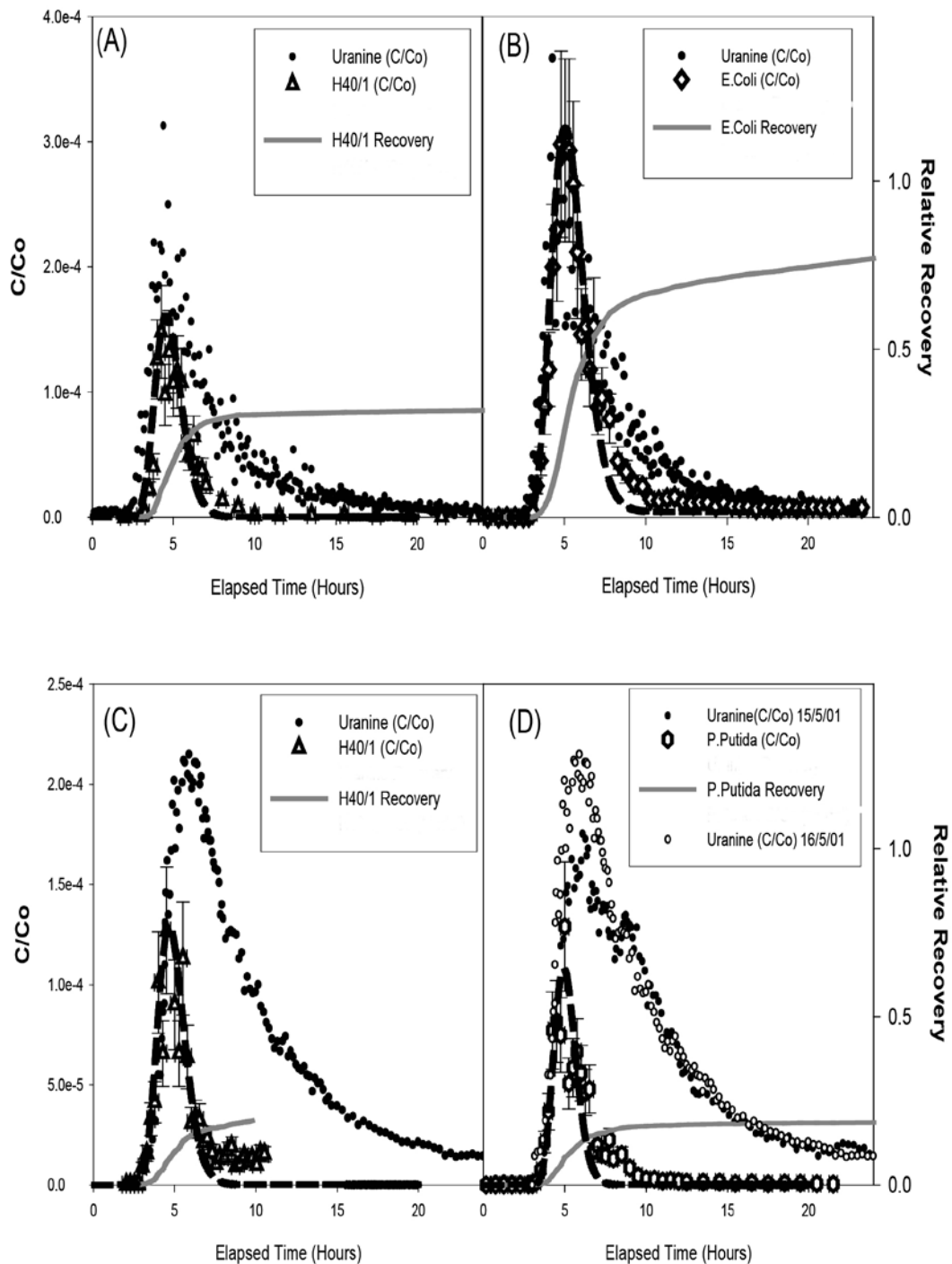


Figure 4: Solute and biocolloid breakthrough curves for Monitoring well B8, Dornach Test Site, Dornach, Germany. Solute breakthrough curves downloaded directly from data logger. *H40/1* & Uranine, 18 July 2000. (B) *E.coli* & Uranine, 17 July 2000. (*E. Coli* Background Conc.: 2.25×10^{-8}). (C) *H40/1* & Uranine, 16 May 2001. (D) *P.putida* & Uranine, 15 July 2000. Irregular signal between 5 and 9 hours after injection due to blockage in fluorimeter. Uranine response from 16 May 2001 presented for comparative purposes and used in subsequent calculations. Modeled microbiological tracer breakthrough curves shown as dashed black lines. Temporal microbiological tracer sampling error ± 0.25 hours. Analytical microbiological tracer error $\pm 25\%$ of measured conc.

The responses observed in tests using *E.coli* and H40/1 contrast with those observed in tests using *P.putida*/H40/1. The value for H40/1, determined from the BTC generated at B8, compares favorably with those determined for tests carried out the previous year, while values for *P.putida* are higher and correspond to more positive zeta potential measurement for this bacterium. Estimated collision efficiencies, determined for H40/1 in tests employing the *P.putida*/H40/1 pair at B7, differ from those determined for B8, with the value estimated for *P.putida* being significantly lower than that determined for B8 (1.8×10^{-3} at B7 vs 7.9×10^{-3} at B8).

Sensitivity analyses of model inputs has revealed variations in porosity, velocity and collector size resulted in significant changes in estimated global collision efficiencies, with changes in collector diameter being particularly noteworthy. For example, a change in collector diameter from 1.5mm to 2.0mm resulted in estimated collision efficiencies for bacterial tracers increasing by over a factor of two; an increase of over 30% was noted for H40/1. Despite these changes, the relative values of collision efficiency remained consistent between microbiological tracers. On the other hand, adjusting the collector diameter to generate a H40/1 collision efficiency at B7, equivalent to that observed at B8 during the *P.putida*/H40/1 tests, fails to generate comparable collision efficiency for *P.putida*; this remains consistently less than H40/1.

Discussion

Comparison of grain size distribution curves for borehole samples, collected from below the test site by Seiler and Müller (2003), with those generated from thicker sequences of silty gravels and sandy gravels observed in outcrop reveals a close correspondence. This suggests that comparable deposits to those observed in outcrop occur at depth. On the other hand the absence of curves resembling those generated from the thin beds of OWG in samples collected at depth is noteworthy, i.e. samples with grain size distribution curves resembling the coarse thin OWG deposits have not been reported. Nonetheless these units are suspected to be present. Their absence in the sampling record is believed to be a consequence of mixing of OWG samples with enclosing finer-grained materials, obtained during well installation using percussive drilling. Further evidence for the occurrence of thin highly permeable OWG units at depth is provided by the rapid arrival and recovery of virtually all solute tracer observed in 10cm to 50cm thick horizons in tracer tests employing mobile downhole fluorometer at B8 (Flynn et al., 2005), and in the extremely gentle hydraulic gradients encountered across the test site (<0.001); transport through medium sands at velocities comparable to those observed would require gradients at least 50 times those noted on site. These findings provide strong evidence that tracer mass transport below Dornach is dominated by groundwater flow through highly permeable units. Furthermore, depth-specific comparative tracer testing, completed prior to the tracer test program reported, confirmed bacteriophage to arrive through the same horizons as the solute, while experiencing low levels of attenuation (Flynn, 2003). The low attenuation rates are again consistent with coarse-grained highly permeable beds rapidly delivering tracers to observation wells.

Tracer responses observed using Uranine at both B7 and B8 in tests completed on successive days suggest that flow dynamics did not change significantly at the test site over the 48 hour intervals in which the tests were carried out. On the other hand, the inability of ADE models of solute tracer BTCs to generate suitable transport parameters to allow simulation of microbiological tracer breakthrough further underscores the benefit of considering

(cumulative) relative recovery, while focusing on using microbiological BTCs alone to simulate responses.

Relative recoveries indicate that while the aquifer underlying the Dornach Test Site has limited capacity to attenuate the microbiological tracers employed, the degree of attenuation varied considerably between tracer types, notably between *E.coli* and H40/1. By contrast, the close correspondence between collision efficiencies estimated for *E.coli* and H40/1 from BTC responses at both B7 and B8 is consistent with their comparable zeta potentials. The comparable collision efficiencies, in turn, suggest that the difference in recovery observed between the two tracers can be attributed to the smaller size of H40/1, and the release of *E.coli* during the latter part of the test monitoring period. As a corollary to this point, the comparable collision efficiency estimates determined for both (similarly-charged) tracers at both B7 and B8 points to relatively homogeneous geochemical conditions encountered by the two microbiological tracers along flow paths connecting injection and observation wells at the time of tests. It also underscores the importance for particle surface in influencing attenuation rates.

The surfaces responsible for the removal of the *E.coli* and H40/1 remain to be definitively identified, although findings from elsewhere point to candidate materials. Studies completed Ryan et al., (1999) reported the presence of iron oxides as playing a significant role in bacteriophage attenuation in the siliciclastic aquifer at Cape Cod, MA. Iron oxides are also suspected to have influenced microbiological tracer responses at Dornach, as evidenced by both borehole logs and the outcrop observations of localized staining. In the absence of comparable microbiological tracers, first principles suggest that attenuation rates at Dornach may be lower due to the higher pH of the carbonate-buffered groundwater. (Experimental investigations carried out by Flynn et al. (2004b) demonstrated both calcite and quartz have very limited capability to remove H40/1 under comparable hydrochemical and hydrodynamic conditions to those observed at the site.)

The estimated collision efficiencies for H40/1, determined at B8 during tests following injection of *P.putida*, suggest consistent flow paths connecting the tracer injection well compared to those following the injection of *E.coli*; a comparable response was observed in repeated solute tracer testing with a mobile downhole fluorimeter (Flynn et al., 2005). Consequently the lower recovery of *P.putida*, compared to *E.coli* and despite its comparable size, is believed to arise because of differences in the microorganism's surface properties. This leads to greater removal following collision with aquifer materials. Overall, responses at B8 show that microorganism collision efficiencies are roughly proportional to their zeta potentials. This further indicates that electrostatic processes play a significant role in attenuating microbiological tracers passing between the injection well and the monitoring well.

The contrast in H40/1 and *P.putida* responses observed at B7, relative to those at B8, cannot be explained by the same particle (microorganism)-fixed surface(grain) interactions. The lower estimated collision efficiency for the H40/1, determined from responses in tests following injection of *P.putida*, suggest that attenuation mechanisms differ from those determined from tests completed the previous year (employing H40/1 with *E.coli*). Moreover, unlike tests completed the previous year, use of filtration theory fails to generate collision efficiencies for both H40/1 and *P.putida* that can be shown to be consistent with those determined for B8 by adjusting aquifer physical properties of the system alone (grain size, porosity). This points to the influence of geochemical heterogeneity, corroborated by

observations in outcrop and in samples collected from test site boreholes, where the occurrence of localized staining reflects spatially variable conditions on collector surfaces. The precise nature of the interactions giving rise to reduced collision efficiency values remains ambiguous. However, microbiological tracer zeta potential and contact angle measurements tentatively suggest a reduced relative importance for electrostatic interactions, with hydrophobic interactions possibly having a more significant influence.

The difference in collision efficiencies, arising due to suspected interactions with collector surfaces having different chemical characteristics, suggests that the properties of the units delivering tracer from the injection well to the observation well were not the same and that different flow paths connected the injection well with B7 during the tests carried out one year apart. This change in flow paths is consistent with analyses of solute breakthrough responses by Mallèn et al. (2005) who showed that different (intrinsic) dispersivity values were necessary to simulate the solute BTCs observed at B7 on each occasion. Furthermore the findings corroborate the results of tests carried out in similar (fast flowing) sand and gravel aquifers in Switzerland where Kennedy et al. (2001) observed tracer breakthrough at selected observation wells in the Bois du Finges sand and gravel aquifer during one tracer test, but failed to observe breakthrough at the same wells during a second test. Similarly, Flynn (2003b) noted comparable responses in a slower flowing sand and gravel aquifer at the Kappelen Test Site (also in Switzerland).

Study results highlight the complexity of groundwater flow regimes operating at Dornach. The comparable responses have been observed at other sand and gravel aquifer test sites in Central Western Europe (Rossi et al., 1994; Kennedy et al., 2001, Flynn, 2004b) suggest that the conditions noted at Dornach occur widely in mountain front sand and gravel aquifers across the region. Variations in flow velocities and directions can give rise to contrasting microorganism attenuation rates. These may arise due to differences in microorganism properties, groundwater hydrodynamics or aquifer (physical and/or compositional) heterogeneity. Use of microbiological tracers with contrasting dimensions and surface chemistry characteristics, coupled with filtration theory, provides a means of further constraining these variables and evaluating the contributions made by compositional and textural aquifer heterogeneity.

From a public health perspective, the rapid microorganism transport rates and low levels of attenuation observed in the tracer tests appear to conflict with findings of raw water quality surveys. Studies, such as that completed by Diston et al. (2015), which noted sporadic detections of low concentrations of viruses in Swiss sand and gravel aquifers, have suggested long groundwater residence times contribute to groundwater attenuation. The short tracer residence times observed at Dornach and other sites suggest otherwise. Nonetheless, analyses for indicators of pathogenic microorganism contamination in the raw groundwater in the aquifers studied failed to detect extensive and/or persistent contamination, despite the occurrence of widespread pollutant sources. This points to the influence of effective microorganism natural attenuation mechanisms.

Examination of geological conditions can contribute to better understanding this paradox. Outcrop studies, such as those completed at the gravel pits at Dornach, and at comparable locations in Switzerland (See Huggenberger and Regli, 2006), reveal the coarse open framework gravels to be overlain by finer grained materials with effective grain size diameters up to two orders of magnitude smaller. Filtration theory predicts that a decline in grain size of one order of magnitude for the system investigated at Dornach will lead to a 1-2 order of magnitude increase in deposition constant, and thus significantly greater attenuation

rates. This, coupled with longer residence times in finer-grained materials, suggests that the finer-grained materials overlying coarser, more transmissive units offer significant levels of protection to raw water quality. Further protection may be afforded should OWG units laterally pinch out/grade into finer-grained deposits, in a manner akin to that noted for preferential flow paths in laboratory studies reported by Wang et al. (2014). Conversely, by-passing these finer grained protective layers, or their removal by excavation, places groundwater quality at higher risk of contamination. Microbiological contamination of groundwater at Dornach in the early 1970s was traced back to leakage from buried sewers. Similarly, the water borne disease outbreak documented in porous deposits at La Neuveville in Switzerland (Maurer and Sturcheler, 2000) was again linked to leakage from wastewater utilities (R. Koezel, BAFU, pers. Comm).

In terms of protecting individual groundwater supplies, the utility of attenuation parameters determined under passive gradient testing provide the basis for development of well head protection models, where the velocity fields can prove variable with distance from abstraction points. Findings from investigations completed at Dornach suggest that incorporation of groundwater velocity variation in porous media using the filtration theory, coupled with selection of appropriate collision efficiencies, provides a defensible means of simulating the removal enteric microorganism contamination in porous aquifers under forced gradients. However, application of the method requires appropriate geological characterization. Results from this study highlight the benefits of employing near surface outcrop observations, and tracers with contrasting but well characterized properties to improve our understanding of contaminant fate and transport in the subsurface.

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