GLOBAL WATER PATHOGEN PROJECT

PART TWO. INDICATORS AND MICROBIAL SOURCE TRACKING MARKERS

EVALUATION OF SUBSURFACE MICROBIAL TRANSPORT USING MICROBIAL INDICATORS, SURROGATES AND TRACERS

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Summary

The goal of this chapter is to provide the readers with useful information that can help with evaluating the microbial contamination risk of groundwater from faecal sources, determining safe setback distances and selecting suitable sites for wastewater reclamation.

Estimating microbial contamination risk in groundwater from faecal wastewater disposals requires knowledge of faecal pathogen loading, microbial attenuation in subsurface media and their transport pathways, which are the essential information required for Quantitative Microbial Risk Assessment (QMRA).

The chapter will outline the available indicators, surrogates, mathematical models, laboratory and field methods that are used for evaluating microbial subsurface transport, and summarise the current knowledge on the capacity of subsurface media in attenuating microbial contaminants. The usefulness and limitations of the exiting tools will be discussed to guild the end-users to find the right decisions about management, treatment and monitoring.

A number of useful tracers (salts, dyes, DNAs and emerging chemical tracers) for establishing flow connection, identifying and tracking the sources/pathways of faecal contamination in groundwater are described. Microbial tracers, microspheres and novel “micro mimics” (biomolecule-modified particles) to determine microbial attenuation and transport in porous media are discussed. Information on their applicability and representativeness are given, including their enumerable concentration range by detection methods.

Mathematical models are given to describe processes involved in microbial transport in subsurface media, including advection, dispersion, degradation (persistence/inactivation), attachment, detachment, straining and colloidal associated transport.

Laboratory methods are described for characterising the physiochemical properties of (bio)colloids, soil and aquifer media, determining microbial inactivation rates using incubation tests, examining particle attachment/detachment between water-solid interface in batch tests, and studying mechanisms and processes of microbial transport in porous media using column experiments. Relative attenuation of pathogens, indicators and surrogates determined from column studies in various porous media is summarised.

Field studies of microbial transport are discussed in investigating the impacts of preferential flow and heterogeneity of porous medium and (bio)colloid properties on microbial transport. This is followed by a summary of microbial removal rates and transport parameter values derived from field studies using indicators, surrogates and pathogens in soils, vadose zones, and groundwater (with links to other chapters). Based on microbial removal rates derived from field studies, suitable sites for wastewater reclamation are recommended.

One section presents model tools for calculation of protection zones/setback distances and for including river bank filtration (RBF) as part of QMRA. For RBF these tools are QMRAspot and QMRAcatch.

Finally, a selection of field studies covering developing and developed countries are described. These studies encompass relatively homogenous fine-grain aquifers (sandy aquifers) and heterogeneous aquifers (alluvial sandy gravels, karst, fractured rocks), focusing on cases of groundwater contamination by wastewater disposals.

Evaluation of subsurface microbial transport using indicators, surrogates and tracers

1.0 Scope

The main intention of this chapter is to explain and describe available indicators, surrogates and tracers, as well as mathematical models for estimating the fate and transport of pathogenic microorganisms (pathogens) in the subsurface. "Subsurface" means everything below ground surface including soils, vadose zone and groundwater. Groundwater constitutes 97% of global freshwater and is the most important source of drinking water in many regions of the world. Typically, groundwater is of more stable quality and better microbial quality than surface waters. Because of their open character, surface waters are much more exposed to faecal contamination sources than groundwater. Major faecal contamination of surface water comes from wastewater discharges and runoff of manure from land. Nevertheless, in the presence of faecal contamination sources, such as farm animal manure and wastewater from leaking sewers and septic tanks, groundwater may readily be contaminated; disease outbreaks from contaminated groundwater sources are reported in countries at all levels of economic development (Howard et al., 2006). An appreciable number of serious health concerns may occur as a result of the chemical contamination of drinking water, but the great majority of evident water-related health problems are the result of microbial (bacterial, viral, protozoan or other biological) contamination (WHO, 2011).

In addition to leaking sewers, septic tanks, and pit latrines, in areas suffering water scarcity, activities like managed aquifer recharge (MAR) with treated wastewater to supplement groundwater sources, also introduce pathogens. Despite all these contamination hazards for groundwater, when keeping enough distance (setback) between a faecal contamination source and a groundwater well for drinking water purposes, the groundwater may be sufficiently protected because of the natural processes in the subsurface that attenuate faecal contamination. Microorganism concentrations in groundwater are reduced by inactivation or die-off of the microorganisms, which is time dependent, and by attachment and filtration of the microorganisms to the solid surfaces of the soil particles,

implying their removal from the aqueous phase. In other words, the subsurface may act as a protective barrier against pathogens from faecal contamination by natural treatment. If natural treatment is inadequate then additional treatment, like disinfection, is required. Note that to abstract safe drinking water, pathogen concentrations from a faecal source need to be reduced many orders in magnitude (3 to 9 orders; a factor of one thousand to one billion). In order to be sure of adequate setback distances, one needs to know the presence/location/size of faecal sources as well as pathogen concentrations in these sources, and how much natural treatment in the subsurface takes place over a given setback distance. Information on faecal sources can be collected by means of sanitary surveys and analysing water samples from the faecal sources. To estimate natural treatment in the subsurface a range of activities is needed. First, one needs to determine groundwater flow (travel times, preferential flow paths, dispersion, dilution). Flow tracers serve that purpose (section 3). Monitoring the abstracted groundwater for pathogens is hampered by their concentrations being commonly below detection level (but may still be unacceptably high), being too technical, being potentially harmful. Therefore, refuge is taken to analysing faecal indicator organisms that resemble pathogens in their fate and transport characteristics, but are available at higher concentrations, and are harmless. Typical examples of faecal indicators are bacteriophages for human pathogenic viruses and *E. coli* for pathogenic bacteria (section 3). Table 1 gives an overview of the indicators, surrogates and tracers included in this chapter and their usage. Given the knowledge about the faecal source and the fate of a pathogen in the subsurface, mathematical transport models come into play to predict pathogen concentrations at the well and/or to calculate the setback distance that is required to produce safe drinking water, in which the pathogen does not exceed a certain risk level, based on a defined health-based target (WHO, 2011).

Table 1. Indicators, surrogates and tracers included in this chapter

Group	Category	Section	Source	Usage
Microbial indicators/models	F-specific RNA bacteriophages, MS2, PRD1, *E. coli*, spores	3.2	Present in human effluent	To indicate contamination from human effluent and determine microbial attenuation and transport
Synthetic surrogates	Unmodified microspheres, biomolecule-modified microspheres	3.3	Introduced	To mimic microbial attenuation and transport
Flow tracers	Chloride, bromide, fluorescent dyes, synthetic DNAs	3.4	Usually introduced	To establish hydraulic connection, determine flow velocity and dispersion. DNA tracers can be also used for tracking pathway of contamination and determine preferential flow of colloid transport
Emerging chemical tracers/indicators	Caffeine, artificial sweeteners, carbamazepine, X-ray contrast media	3.5	Present in human effluent	To indicate contamination from human effluent

Much of the information presented in this chapter relies on literature reviews on research in this area. For in-depth technical details, the reader is referred to those reviews. A number of reviews focussed on fate and transport in the subsurface of viruses (Harvey and Harms, 2002; Harvey and Ryan, 2004; Jin and Flury, 2002; Pang, 2009, Schijven and Hassanizadeh, 2000), bacteria (Foppen and Schijven, 2006; Jamieson et al., 2002; Harvey and Harms, 2002; Pang, 2009) and protozoa (Harvey and Harms, 2002; Pang, 2009; Park et al., 2012; Tufenkji et al., 2006). Other reviews consider microorganisms in general (Bradford et al., 2013; 2014; Bradford and Torkzaban, 2008; Ginn et al., 2002; McDowell-Boyer et al., 1986). Mostly, the reviews summarize data on indicator organisms, particularly bacteriophages, *E. coli*, faecal coliforms, and bacterial spores. In the subsurface, microorganisms are transported with the water flow (advection) and are dispersed with travel distance. Their inactivation in the subsurface and filtration processes determine to what extent they are removed from the water phase for which a number of reviews provide mathematical model descriptions (Bradford et al., 2013; Foppen and Schijven, 2006; Ginn et al., 2002; Harvey and Harms, 2002; Jin and Flury, 2002; McDowell-Boyer et al., 1986; Pang, 2009; Ryan and Elimelech, 1996; Sen and Khilar, 2005; Tufenkji et al., 2006) and model parameter values (Foppen and Schijven, 2006; Pang, 2009; Park et al., 2012). Bradford et al. (2013; 2014) and Bradford and Torkzaban (2008) reviewed modelling of transport of microorganisms in the vadose zone. Jamieson et al. (2002) summarized information with respect to the persistence of faecal bacteria in soil waste systems and their transport to tile drainage water. Pachepsky et al. (2006) and Unc and Goss (2004) focus on persistence of bacteria in manure prior to, and when applied to, land. Note that inactivation (or die-off) is complementary to persistence (see chapters on persistence). Inactivation is a

removal process, persistence is not.

2.0 Microbial Removal in the Subsurface Media

2.1 Introduction

While subsurface media naturally filter microbes and mitigate microbial contamination, their capabilities in microbial removal vary widely according to the type of medium. Microbial removal in this context is defined as the logarithmic reduction in microorganism concentration in subsurface media due to the processes that remove the microorganisms from the groundwater or soil-water phase. This can be by means of inactivation or die-off, whereby infectious microorganisms actually disappear, and/or by attachment to the solid surface of the soil grains. The latter process may be reversible. Microbial removal rates denote removal per time (temporal removal rate) or per meter transport distance (spatial removal rate). This section focusses on spatial removal rates. To assess water contamination risks, estimate setback distances and select wastewater reclamation sites, it is crucial to gather information about the microbial removal rates in subsurface media. This information will help improve resource management and groundwater contamination monitoring (see technology chapters).

Data from field studies are the most applicable to resource management. Comparatively, less information about microbial transport is available from field studies than from column studies performed in the laboratory. However, laboratory column studies often do not represent field conditions accurately, because microbial transport is hugely affected by the physical and chemical properties that are associated with media heterogeneity and transport scale. These factors are difficult to reproduce in the laboratory. Furthermore, repacking tends to reduce the macropores of a subsurface medium. Pang (2009) compared 6 pairs of field and laboratory column studies that had been undertaken using sand, fine gravel and limestone media, and found that microbial removal in the laboratory column studies was 1–3 orders of magnitude greater than that determined from field studies.

By analysing a large body of published data obtained from field experiments and large undisturbed soil cores, Pang (2009) established a comprehensive database on the removal rates of viruses, bacteria and protozoa in a variety of subsurface media under different environmental conditions. The removal rates provided in the database are presented in a simple form. It thereby enables their easy use by practitioners, namely, regulators, environmental managers, utility staff, consultants and researchers, as they tackle environmental management problems.

For most practitioners, the overall reduction of microbial contaminants in subsurface media is of foremost interest. The microbial concentrations measured down-gradient from a contamination source are the consequences of all reduction processes, including irreversible attachment, inactivation or die-off, straining and dilution. The removal rate (λ) given in Pang (2009) has lumped the effects of all of these reduction processes, because from the presented information in the literature it was not always possible to separate the effects of the individual processes. A spatial removal rate measures the $\log_{10}$-reductions in the microbial concentrations per unit of distance travelled, i.e., $\log_{10}$/m, which is expressed simply as log/m in this section. The database from Pang (2009) is summarised below. Detailed information on the data analysis and individual source references can be found in Pang (2009).

2.2 Soils

The microbial removal rates for soils were derived from field studies that used soil depths of <1 m and intact soil cores that were >0.4 m long. Hence, these removal rates should not be extrapolated to depths of >1 m, and for these depths, the removal rates for the vadose zone media (link to glossary) should be considered. Most of the experimental data used were derived from soils that were impacted by effluent irrigation or discharge. The following commentary summarises the microbial removal rates for soils that were given in the database of Pang (2009). Tables 2 and 3 summarise the virus and bacterial removal rates in different soils.

Table 2. The efficiencies of virus removal in different soils

Soil Type	Contamination Source	Microbe	Removal Rate (Log_{10}/m)		
			Mean	Minimum	Maximum
Allophanic soil	Dairy shed effluent	*Salmonella* phage	Complete removal		
Clay loam	Dairy shed effluent	*Salmonella* phage	1.8	1.59	2.15
Clayey soil	Dairy shed effluent	*Salmonella* phage	0.97	0.12	2.08
Deep silt loam	Dairy shed effluent	*Salmonella* phage	1.99	1.56	2.56
Fine - very fine sand	Sewage	PRD1	9.19	5.02	13.68
Fine sandy loam	Sewage sludge	Poliovirus	5.26	4.97	5.54
Fine sandy loam	Dairy shed effluent	*Salmonella* phage	2.98	2.4	3.28
Loamy sand	Microbial tracer	*Salmonella* phage	3.76	2.74	4.87
Pumice soil	Dairy shed effluent	*Salmonella* phage	16.61	15.75	17.46
Recent sandy soil	Dairy shed effluent	*Salmonella* phage	2.46	2.08	2.89
Shallow silt loam over gravel	Dairy shed effluent	*Salmonella* phage	1.98	0.99	2.53
Silt loam	Dairy shed effluent	*Salmonella* phage	2.3	2.07	2.69
Silty clay loam	Dairy shed effluent	*Salmonella* phage	2.8	1.87	4.18
Silty sands & gravel	Sewage	f2 bacteriophage	2.19	1.31	2.86

Summarized by using data from: Pang, 2009

Table 3. The efficiencies of bacterial removal in different soils

Soil Type	Contamination Source	Microbe	Removal Rate (Log_{10}/m)		
			Mean	Minimum	Maximum
Allophanic soil	Dairy shed effluent	Fecal coliforms	5.48	5.22	5.75
Allophanic soil	Dairy shed effluent	*E. coli*	5.34	5.04	5.63
Allophanic soil	Dairy shed effluent	Enterococci	5.16	5.05	5.28
Bare sandy loam	Cow manure	Fecal coliforms	2.41	NR	NR
Clay	Septic tank effluent	Fecal streptococci	6.04	NR	NR
Clay	Septic tank effluent	Fecal coliforms	3.67	NR	NR
Clay loam	Dairy shed effluent	Fecal coliforms	2.64	2.08	3.17
Clay loam	Cow manure	Fecal coliform	0.46	NR	NR
Clay loam	Septic tank effluent	Fecal streptococci	1.75	NR	NR
Clay loam	Septic tank effluent	Fecal coliforms	0.81	NR	NR
Clayey silt loam	Cow manure	*E. coli*	0.54	0.42	0.65
Clayey silt loam	Dairy shed effluent	Fecal coliforms	0.55	0.4	0.69
Clayey silt loam	Dairy shed effluent	Enterococci	0.27	0.2	0.33
Clayey soil	Dairy shed effluent	Enterococci	0.79	0.72	0.86
Clayey soil	Dairy shed effluent	*E. coli*	0.34	0	0.69
Clayey soil	Dairy shed effluent	Fecal coliforms	0.41	0	0.83
Deep silt loam	Dairy shed effluent	Fecal coliforms	4	0.12	6.25
Fine sandy loam	Dairy shed effluent	Fecal coliforms	9.34	8.88	9.56
Loam	Septic tank effluent	Fecal streptococci	5.5	NR	NR
Loam	Septic tank effluent	Fecal coliforms	4.89	NR	NR
Loam	Dairy shed effluent	Fecal coliforms	0.75	0.43	1.06
Loamy sand	Septic tank effluent	Fecal coliforms	4.02	1.38	6.66
Loamy sand	Septic tank effluent	Fecal streptococci	3.72	1.37	6.07

Marshland	Sewage	Fecal coliforms	2.38	1.19	3.88
Marshland	Sewage	*E. coli*	1.13	0.99	1.28
Pumice sand soil	Dairy shed effluent	Fecal coliforms		Complete removal	
Recent sandy soil	Dairy shed effluent	Fecal coliforms	2.34	1.96	2.77
Sandy loam	Septic tank effluent	Fecal streptococci	3.87	2.24	5.17
Sandy loam	Septic tank effluent	Fecal coliforms	3.7	2.63	5.13
Sandy loam	Dairy shed effluent	Fecal coliforms	2.78	2.24	3.31
Shallow silt loam over gravels	Dairy shed effluent	Fecal coliforms	4.04	2.42	6.49
Silt loam (25 to 40 mm/h)	Dairy shed effluent	Fecal coliforms	6	4.27	7.14
Silt loam (5 mm/h)	Dairy shed effluent	Fecal coliforms	2.47	2.27	2.79
Silt loam (flood irrigation)	Dairy shed effluent	Fecal coliforms	4.11	NR	NR
Silty clay loam	Dairy shed effluent	Fecal coliforms	3.61	2.77	5.16
Silty clay/clay	Tracer	*E. coli*	0.34	0.32	0.36
Silty clay/clay	Septic tank effluent	Fecal streptococci	2.76	NR	NR
Silty clay/clay	Septic tank effluent	Fecal coliforms	2.44	NR	NR
Silty sands and gravel	Sewage	Fecal coliform	8.28	NR	NR
Silty sands and gravel	Sewage	Fecal streptococci	4.81	2.31	8.58
Stony silt loam	Cow manure	Fecal coliform	2.48	1.61	2.69
Vegetated sandy loam	Cow manure	Fecal coliform	1.6	NR	NR

Summarized by using data from: Pang, 2009; NR: Not Reported

Generally for every $\log_{10}$ reduction in microbial concentration, it takes 0.2–0.6 m depth in most soils but only 0.1 m in very fine sand and pumice sand soils. However this can vary from 0.2 to 4 m in clay soils. Microbial removal rates for soils are one to a few log/m for most soil types. It ranges from complete removal for allophanic and pumice sand soils and as low as 1 $\log_{10}$/m for clayey soil. Allophanic and pumice sand soils have the greatest capacity to remove both bacteria and phages, because allophanic clays carry a net positive charge at a pH of <6 and they have very large surface areas—characteristics that foster the attachment of negatively charged microbes. Allophanic clays are found in volcanic ash and in the weathering products of volcanic rocks, greywacke and schist.

In terms of microbial removal rates, volcanic soils are followed by fine sandy loam, sandy loam and loamy sand soils. While fine sandy loam removes bacteria very effectively, which probably occurs through straining, it is relatively ineffective at removing phage viruses. Silt loam, and shallow and deep silt loams are moderately proficient at removing microbes.

Microbial removal is worst in clayey soils and clay loam. While clay particles filter microbial particles very effectively under conditions of ideal matrix, clay soils can shrink and crack under field conditions, which leads to the development of macropores and preferential flow paths.

Removal rates vary more in soils that contain clay and gravels (clayey soil, silty clay loam, clay loam, silt loam-over-gravels and deep silt loam) than in fine-textured and volcanic soils (silt loam, fine sandy loam, recent sandy soil, allophanic soil and pumice sand soil).

2.2.1 Effect of soil structure

Under field conditions, soil structure (i.e., macropores) often has a greater impact on microbial removal than the soil texture. A clay soil core that has many cracks and channels may aid microbial transport as opposed to a sandy soil core that has a more homogenous pore structure. Soil structure may change with the seasons, which is particularly pertinent to soils with a higher clay content in the topsoil, because shrinkage cracks can form during summer but can close up during wet seasons.

Soil structure or heterogeneity largely determines its microbial leaching potential. The microbial leaching potential of a soil is inversely related to the volume of irrigation water required to elute the maximum concentration. Irrigation of a very small amount of water on structured clay soils could lead to rapid leaching of microbial contaminants through cracks. Rapid microbial leaching immediately after effluent irrigation is often observed in structured clayey soil, clayey silt loam, and clay loam. Conversely, microbial leaching from pumice sand and other sandy soils requires relatively larger volumes of irrigation water. The microbial leaching potential of soils is in the descending order of structured clay soils, silt over gravels, silt soils, sandy soils, pumice sand soil and allophanic soils (Figure 1).

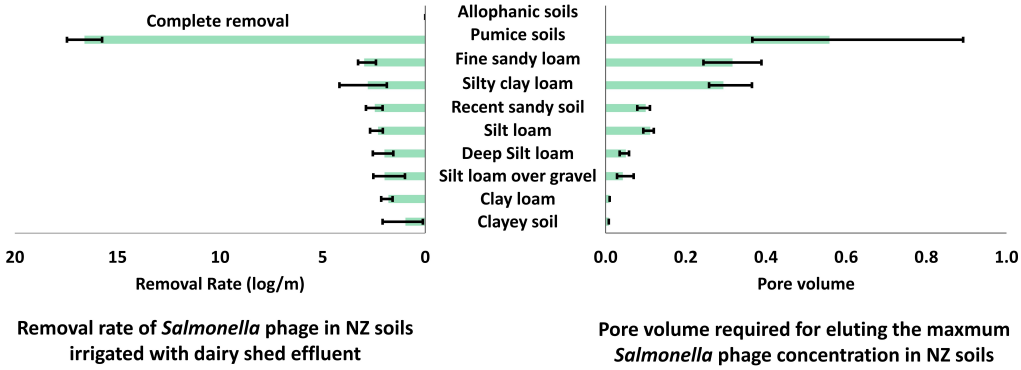

Figure 1. Removal rates and leaching potential of Salmonella bacteriophages in New Zealand soils under dairy shed effluent irrigation (summarized by using data from Pang, 2009).

2.2.2 Effect of irrigation practices

For a particular soil, the experimentally determined microbial removal rates under flood irrigation are lower, but less variable, than those determined under spray irrigation. This is because during flood irrigation the drainage of water from the soil greatly exceeds the soil moisture, whereas during spray irrigation, the amount of water applied may or may not exceed the soil moisture deficit. This depends on the season, the irrigation method used, the irrigation rate and the uniformity of application.

2.2.3 Comparison of viruses and bacteria

For a specific soil, the faecal coliform removal rate tends to be higher than the bacteriophage removal rate, but they are within the same order of magnitude. Faecal coliforms, *Escherichia coli*, *streptococci* and *enterococci* have similar removal rates.

2.2.4 Remarks

Information about microbial removal rates in soils can be used to select suitable soil media for effluent disposal, including the selection of backfill materials in septic tank disposal trenches, soil treatment systems, effluent infiltration basins and field sites for effluent discharge and irrigation. The most suitable soil media for effluent disposal are volcanic soils followed by sandy soils. Clayey and gravely soils are not desirable for effluent disposal.

Nutrients are commonly recycled to fertilise pastures, forests and crops by applying domestic effluent and animal manures to land. The efficiency of microbial removal in soils could be enhanced by appropriate management practices. For example, microbial leaching could be reduced by reducing irrigation rates, by allowing periodic drying and wetting, and by disrupting the macropores in soils using periodic tillage, especially in vegetated soils.

2.3 Vadose Zones

Vadose zones (link to glossary) are the unsaturated zones that lie between the region that is below the soil and above the groundwater table. As soil studies analysed in Pang (2009) extended to a depth of 1 m below ground (refer to section 2.2), an arbitrary value of 1 m was used in Pang (2009) for the top of the vadose zone. Removal rates are generally lower in vadose zone media than in soils.

Fewer studies have investigated microbial removal in vadose zones compared with the number of studies on microbial removal in soils and groundwater. The removal rates estimated by Pang (2009) for vadose zones (Tables 4 and 5) were derived from studies of wastewater or sewage effluent infiltration basins, and infiltration experiments using tracer solutions, septic tank effluent and animal effluent, and these are summarised next.

Table 4. The efficiencies of virus removal in different vadose zone media

Vadose Zone Media	Contamination Source	Microbe	Removal Rate (Log10/m)		
			Mean	Minimum	Maximum
Bassenden Sand with high silica content	Sewage	Echoviruses 24	1.08	NR	NR
		Echoviruses type 11	0.37	NR	NR
		Poliovirus	0.95	NR	NR
		Coxsackieviruses B5	0.95	NR	NR
		Coxsackieviruses B4	0.48	NR	NR
		Enteroviruses	0.26	NR	NR
Coarse sand and gravels with clay lenses	Sewage	MS2	NR	0.05	NR
		PRD-1	NR	NR	0.59
Coarse sand, and fine gravel	Wastewater	Poliovirus	0.29	0.23	0.36
Fine-coarse sand	Wastewater	PRD-1	1.52	0.94	2.09
		MS2	0.95	0.46	1.43
Fractured clayey till	Microbial tracer	PRD-1	1.59	NR	NR
Pumice sand	Sewage	F-RNA phages	4.83	NR	NR
Sand (d=0.18 mm)	Wastewater	Coliphage	0.15	NR	NR
Sandy gravel and coarse sand with clay	Sewage	MS2	0.53	0.12	NR
Weathered and fractured granite	Microbial tracer	MS2	0.89	NR	NR

Summarized by using data data from: Pang, 2009; NR: Not Reported

Table 5. The efficiencies of bacterial removal in different vadose zone media

Vadose Zone Media	Contamination Source	Microbe	Removal Rate (Log10/m)		
			Mean	Minimum	Maximum
Bassenden Sand with high silica	Sewage effluent soakage basins	Fecal coliform	0.53	NR	NR
Coarse gravels	Septic tank effluent soak holes	Fecal coliforms	0.44	0.27	0.5
Fissured chalk	Sewage discharge through soakage	Fecal coliforms	0.32	0.31	0.36
Fissured chalk	Sewage discharge by drainage	Fecal coliforms	0.16	0.14	0.19
Pumice sand,	Septic tank effluent	Fecal coliforms	2.66	NR	NR
Sand (d=0.18 mm)	Sewage effluent groundwater recharge	Fecal coliform	0.84	NR	NR
Silty clay loam	Leaking of a deep pit of pig manure.	Fecal streptococcus	0.88	NR	NR
Very fine uniform dune sands	Wastewater infiltration basins	Fecal coliforms	NR	0.52	NR
Very fine uniform dune sands	Wastewater infiltration basins	Fecal streptococci	NR	0.45	NR

Summarized by using data from: Pang, 2009; NR: Not Reported

2.3.1 Overview

In general, for every $\log_{10}$ reduction in microbial concentration in vadose zone media, it would need 1–7 m transport distance for most media, exception for pumice sand (0.2–0.4 m). The estimated microbial removal rates for vadose zone media mostly range 0.1–1.0 $\log_{10}$/m for clay and silt, sand, sandy gravels, coarse gravels, fractured chalk and granite, and 1.5–4.8 log/m for pumice sand and clay till, and for sand occasionally. Like soil media, the best vadose zone media for effluent infiltration are pumice sand and uniform sand.

2.3.2 Effect of organic matter

Most of the wastewater infiltration basins studied were in use for many years and their vadose zone media were contaminated by organic matter. Under such conditions, viruses and bacteria in the wastewater may be associated with organic matter. Microorganisms in the effluent have to compete with an excess of like-charged organic matter for attachment to solid surfaces resulting in a greater transport.

2.3.3 Remove rate comparison

For the same media, the removal rates for viruses and virus indicators (phages) in vadose zones are in the same order of magnitude as that for bacteria. While the removal rate for MS2 phage tends to be lower than that for PRD1 phage, at low infiltration rates the removal rate of MS2 phage could be slightly higher than that of PRD1 phage, because PRD1 survives longer than MS2 (Gerba et al., 1991). In addition, although MS2 is conservative in hydrophilic media, it attaches to hydrophobic media (Farkas et al., 2014; Burbery et al., 2015). This could have some important implications in virus transport in soils as many soils are water repellent (hydrophobic), especially sandy soils under unsaturated conditions. Water repellence results from coating of waxy organic compounds onto soil particles. MS2 would not be a conservative surrogate in water repellent soils and could over-predict virus removal. Human virus removal rates in sand media are estimated to be in the order of enteroviruses < echovirus type 11 < coxsackievirus B4 < coxsackievirus B5 = poliovirus type 2 < echovirus type 24 (Pang, 2009).

2.3.4 Remarks

Microbial removal rates in vadose zones seem to be inversely related to the infiltration rates, which concurs with the findings in soils. This is because a decrease in the infiltration rate would increase the travel time and reduce volumetric water content, which results in a greater influence of inactivation and the air–water interface on microbial removal. This finding may be useful in managing effluent infiltration. Therefore, controlling the infiltration rate could minimise groundwater contamination from effluent disposal by, for example, periodically drying and wetting the infiltration basins or disposal trenches.

Since surface ponding often happens in infiltration basins, microbial transport often occurs under forced hydraulic gradients and the vadose zone media might be close to saturation. However, in contrast to the transport processes in groundwater, which are mainly horizontal within an aquifer layer, the transport processes in vadose zones are mostly vertical and perpendicular to the lithological units. Furthermore, the geochemical and physical conditions within vadose zones tend to be very different compared with those in unsaturated zones, even for the same lithological units. Therefore, removal rates derived from groundwater would probably not be applicable to similar vadose zone media, even when they are close to saturation.

2.4 Aquifers

Many field studies of microbial transport in groundwater have been described in the literature. Pang (2009) estimated the microbial removal rates in a range of aquifer media under a variety of conditions (Tables 6 and 7). The major findings are summarised next.

Table 6. The efficiencies of virus removal in different aquifer media

Aquifer Media	Contamination Source	Microbe	Removal Rate (Log10/m)		
			Mean	Minimum	Maximum
Coarse gravel	Microbial tracer	MS2	0.025	NR	NR
	Sewage	T4 coliphage	0.004	NR	NR
	Sewage	Somatic phages	0.004	NR	NR
	Sewage	F-RNA phages	0.002	NR	NR
	Sewage	ϕX174 coliphage	0.001	NR	NR
Coarse sand	Microbial tracer	ϕX174	0.218	NR	NR
	Microbial tracer	MS2	0.188	NR	NR
Dune sand	River bank filtration	PRD1	0.198	NR	NR
	River bank filtration	MS2	0.187	NR	NR
Fractured clay till	Microbial tracer	MS2	0.705	0.13	1.45
	Microbial tracer	PRD1	0.8	0.1	1.03
Fractured clayey shale saprolite	Microbial tracer	MS2	0.331	NR	NR
	Microbial tracer	PRD1	0.3	NR	NR
Fractured limestone	Wastewater	Somatic phages	0.001	NR	NR
Karst limestone	Microbial tracer	H4 & H40 phage	0.001	NR	NR
Pumice sand	Microbial tracer	MS2	1.849	NR	NR
Sand and fine gravel	Sewage	PRD1	0.348	0.03	1.11
Sand and gravel	Septic tank effluent	MS2	0.392	NR	NR
	Septic tank effluent	ϕX174 coliphage	0.123	NR	NR
	Septic tank effluent	Background coliphage	0.194	NR	NR
	Septic tank effluent	Male-specific coliphage	0.215	NR	NR
	Septic tank effluent	Somatic phages	0.212	NR	NR
	River bank filtration	Somatic bacteriophage	0.024	0.018	0.03
	River bank filtration	F-RNA phages	0.09	0.057	0.167
Sandy gravel	Microbial tracer	MS2	0.099	NR	NR
	Microbial tracer	PRD1	0.095	NR	NR
	Microbial tracer	ϕX174 coliphage	0.151	NR	NR
	Microbial tracer	Poliovirus	0.097	NR	NR
Silty sands and gravel	Sewage	Indigenous enteric virus	0.003	NR	NR
	Sewage	f2 phage	0.002	NR	NR

Summarized by using data from: Pang, 2009; Note: As the removal rates of aquifer media are much lower than those of soils and vadose zone media, three decimal places were presented.

Table 7. The efficiencies of bacterial removal in different aquifer media

Aquifer Media	Contamination Source	Microbe	Removal Rate (Log_{10}/m)		
			Mean	Minimum	Maximum
Coarse gravel	Sewage effluent	*B. stearothermophilus*	0.003	NR	NR
Coarse gravel	Sewage effluent	*E. coli*	0.005	0.004	0.01
Coarse gravel	Sewage effluent	Faecal coliforms	0.003	NR	NR
Coarse gravel	Tracer	B. subtilis spores	0.031	NR	0.045
Coarse gravel	Tracer	*E. coli* J6-2	0.021	NR	NR
Coarse gravel	Tracer	Faecal coliforms	0.003	NR	NR
Coastal sand	Septic tank effluent	Fecal coliforms	0.159	NR	NR
Dune sand	Sewage effluent	Fecal coliforms	0.014	NR	NR
Dune sand	Sewage effluent	Streptococci	0.005	NR	NR
Fine sand	Septic tank effluent	*C. perfringens*	0.024	NR	NR
Fine sand	Septic tank effluent	*Clostridium*	0.044	NR	NR
Fine sand	Septic tank effluent	Coliform	0.05	NR	NR
Fine sand	Septic tank effluent	*E. coli*	0.048	NR	NR
Fine sand	Septic tank effluent	Enterococci	0.025	NR	NR
Fissured chalk	Sewage effluent	Fecal coliforms	0.023	0.004	0.067
Fractured gneiss	Tracer	*E. coli*	0.115	0.087	0.143
Fractured limestone	Sinkhole	*Clostridium*	0	NR	NR
Fractured limestone	Sinkhole	*E. coli*	0.001	NR	NR
Fractured limestone	Sinkhole	Fecal coliforms	0.001	NR	NR
Fractured limestone	Sinkhole	Streptococci	0.001	NR	NR
Gravel and sand	Tracer	*E. coli*	0.004	0.003	0.004
Karst limestone	Creek	Enterococci	0.017	0.001	0.215
Karst limestone	Creek	Fecal coliform	NR	0.052	0.067
Limestone	Pig manure pit	Fecal streptococcus	0.016	0.015	0.017
Pumice sand	Sewage effluent	Faecal coliforms	3.847	NR	NR
Pumice sand	Tracer	*E. coli*	1.54	1.46	1.61
Sand and fine gravel	Sewage effluent	Protozoa	0.43	0.37	0.5
Sandstone	Pig manure pit	Fecal streptococcus	0.041	NR	NR
Sandy gravel	River bank filtration	Aerobic spores	0.145	0.08	0.27
Sandy gravel	River bank filtration	*Bacillus*	0.052	0.015	0.081
Sandy gravel	River bank filtration	*Clostridium*	0.053	0.015	0.126
Sandy gravel	River bank filtration	Fecal coliform	0.102	0.031	0.148
Sandy gravel	River bank filtration	Protozoa	0.07	0.04	0.09

Summarized by using data from: Pang, 2009; NR: Not Reported; Note: As the removal rates of aquifer media are much lower than those of soils and vadose zone media, three decimal places were presented.

2.4.1 Overview

Coarse gravel aquifers, chalk aquifers and karst limestone aquifers have lower microbial removal capacities. Comparatively, pumice sand aquifers, alluvial sand aquifers and highly weathered aquifer rocks containing clay have much greater microbial removal capacities.

For each type of aquifer, the bacteriophage and bacteria removal rates are on the same order of magnitude. In general, for every $\log_{10}$ reduction in microbial concentration in groundwater, it would need a few tens of meters in clean coarse gravel aquifers and a few hundred meters in contaminated coarse gravel aquifers. In contrast, it only needs a few meters in sandy fine gravel aquifers and sand aquifers to achieve one $\log_{10}$ reduction in the microbial concentration.

2.4.2 Effect of organic matter

Even aquifers that are competent at microbial removal can exhaust their microbial removal capability under long-term loading by contaminant sources. This results in contaminant plumes to develop over large distances. The effect of continuous effluent loading on reduced microbial removal was also demonstrated in short-term experiments.

Microbial removal rates tend to be lower in contaminated aquifers compared with uncontaminated aquifers that are comprised of the same aquifer media. For example, contamination of a coarse gravel aquifer with sewage effluent reduces its microbial removal capability by 1-order of magnitude (order of 10^{-3} log/m) compared with its microbial removal capability in an uncontaminated state (order of 10^{-2} log/m). The reduction in the aquifer's microbial removal capability is attributed to the influence of sorbed and dissolved organic matter and other anions in the effluent. As both organic matter and the microbial particles are net negatively charged, they compete for the same sorption sites in the aquifer media thus less electrostatic sorption sites are available for microbial attachment. This phenomenon is designated as blocking.

2.4.3 Effect of oxygen

For the same microorganism, removal rates in anoxic aquifers are relatively lower compared with oxic aquifers due to lower levels of inactivation and adsorption in anoxic conditions. Oxygen is a major regulator of microbial survival. Hence, under oxic conditions, lipid oxidation can change the bacterial membrane structure and function, thereby damaging microbial proteins (Kreier, 2002). In addition, metal oxides are present in the oxidised conditions, which could also enhance microbial removal, because patches of metal oxides on soil grain surfaces act as favourable sites for attachment.

2.4.4 Effect of flow velocity

It was found that virus removal rate in gravel aquifers decreases exponentially with transport velocity (Pang, 2009). This correlation can be simply explained by the reaction time. As transport velocity increases, there is less reaction time available for microbial particles to interact with the aquifer media, resulting in a lower microbial removal.

2.4.5 Spatial pattern

The estimations of spatial removal rates in Pang (2009) were based on the assumption that microbial concentration decreases exponentially with travel distance (x), as assumed in most traditional transport models. The value of the spatial removal rate is interpreted from the slope of linear fit for a log (C_{max}/C) vs. x plot when there are multiple sampling locations down-gradient of the source. C_{max} is the peak concentration observed in the sampling point and C is the input concentration. Examining 87 comparable cases, Pang (2009) found that 70% of the cases showed a better fit with first-order law for data obtained from both uncontaminated and contaminated media. This indicates that an assumption of a constant removal rate, is appropriate for most of the field data analysed. However, 30% of the datasets were better described with a power law, implying reduced removal rates with transport distance. The latter is more pronounced for organically contaminated media, especially in relatively fine aquifer media. Both relationships are apparent at different transport scales.

Unfavourable attachment conditions as a consequence of the presence of organic matter, heterogeneous attachment conditions (caused by heterogeneity in the properties of the microbial contaminants, changes in solution chemistry and detachment) and physical straining (especially when microbial particles are associated with colloids in effluent) may cause divergence from the first-order law predicted from traditional transport models and filtration theory.

First-order functions better describe removal of some microbial species while power functions better describe removal of other species. This is probably a consequence of the heterogeneity among the microbial particles themselves because of variability in type, size, density, charge, strains, survival characteristics, aggregation with colloids.

2.4.6 Implications for groundwater setback distances

Setback distance estimations require knowledge about microbial removal rates. For people who know little about transport modelling, the simple method given below can be used to estimate an approximate setback distance.

Assuming a continuous constant effluent input, the total reduction in microbial concentrations at a steady state can be calculated using the formula:

$$n = ST + H_f\lambda_f + H_s\lambda_s + H_v\lambda_v + L_\alpha\lambda_\alpha \qquad (1)$$

where, n is the total $\log_{10}$ reduction in the microbial concentration between the contaminant source and the receiving water, ST is the log reduction in the microbial concentration in the on-site treatment system itself, H is

the thickness or vertical distance in metres, L is the horizontal distance in metres, and λ is the spatial removal rate (log/m). The subscripts f, s, v and a are for the backfill material in the disposal system (trench or basin), soil in the drainage field, vadose zone and aquifer, respectively. The target level for total microbial reduction (n) depends on the purpose of the receiving water and the initial concentrations of the pathogens in the effluent.

2.4.7 Application to transport models

The spatial removal rates (λ) can be used in transport modelling after converting them to temporal removal rates (k) if the transport velocities (V) are known ($k = \lambda V$). The spatial and temporal removal rates measure the relative reductions in the microbial concentrations per unit of distance and time travelled, respectively. Pang et al. (2005) and Pang (2009) demonstrated that the λ values determined from the slopes of log (C_{max}/C_o) versus distance plots and those converted from model-derived K values from breakthrough curves are very similar. This suggests that the spatial removal rate can be reliably converted to the temporal removal rate if the transport velocity is known.

When these removal rates are applied to transport models, the dilution effect should be subtracted, because the removal rate was derived from a plot of the slope of $\log_{10}(C_{max}/C)$ versus the distance (excluding the injection well). When published data from experiments using nonreactive tracers are available, the plot of the slope of $\log_{10}(C_{max}/C)$ versus the distance for the nonreactive tracers reflects the effect of dilution, and this should be subtracted from the total removal rate. Unfortunately, such data are unavailable in many published field studies. This is especially true for monitoring data collected from actual contamination sites as opposed to those gathered from tracer experiments.

2.5 Conclusion Regarding Subsurface Transport

The subsurface media that are highly effective in microbial removal, therefore, suitable for effluent land disposal, are allophanic soils, pumice sand, fine sand and highly weathered aquifer rocks. In contrast, the subsurface media that perform poorly in microbial removal are structured clayey soils, stony soils, coarse gravel aquifers, fractured rocks and karst limestones. Figure 2 summarises the range of microbial removal rates in subsurface media.

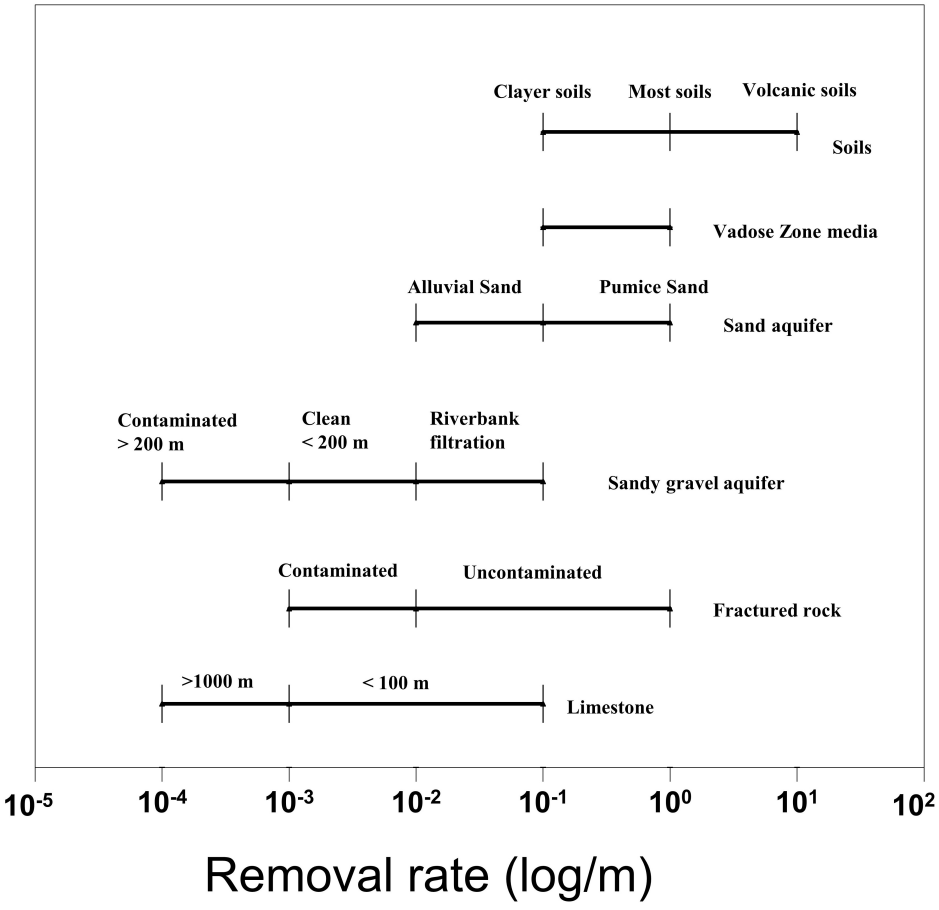

Figure 2. The range of microbial removal rates in subsurface media (summarized by using data from Pang, 2009)

Removal rates are lower for enteroviruses than for other human viruses, for MS2 phage than for other phage species (except for PRD1), for waste-associated microbial species than for those cultivated in the laboratory, and for

sewage-contaminated aquifers than for uncontaminated aquifers. For the same media, virus removal rates are in the same order of magnitude as they are for bacteria, and they can be lower or higher, because they can be removed in association with large colloids.

Removal rates have clear inverse relationships with transport velocity, hydraulic conductivity, hydraulic loading and infiltration rates. Microbial removal rates are specific to the physical and chemical properties of the microbial contaminants and the subsurface media, solution chemistry, transport scale, the type of contaminant source and the contamination duration. When choosing a removal rate from the database provided, readers should try to best match all of the experimental and environmental conditions, particularly the flow rate. The microbial removal rates determined from experiments that involve point sources injected with pulses of microbial solutions will be higher than those determined from area-sources that are under long-term contaminant loading. For a conservative approach, removal rates determined from area-sources under long-term effluent loading should be considered.

Caution should be exercised when extrapolating distances beyond the transport scales that the removal rates are derived from. Removal rates may decrease with distance, especially in fine grain aquifers and aquifers that are under long-term continuous effluent inputs.

Despite the limitations associated with the assumptions and simplifications used in the removal rate estimations, the microbial removal rate database provides useful information about the relative abilities of subsurface media to remove microbial contaminants. The information provided could be used to evaluate the risk of microbial contamination of groundwater under effluent land disposal, to establish safe setback distances between receiving waters and disposal fields, and to select suitable sites for wastewater reclamation and managed aquifer recharge.

3.0 Indicators, Surrogates, Tracers

3.1 Introduction

Studying or monitoring microbial groundwater quality involves time series of water samples taken from a faecal source, from the abstraction well, and from additional monitoring wells. Concentrations of solutes (salt, dye) provide information on water flow. Microbial data may provide information on pathogen removal. In this regard, water samples can be analysed for naturally present solute tracers and microorganisms, or for those intentionally injected at some point at high enough concentration for a detailed study of their breakthrough at monitoring and abstraction wells. Naturally present tracers and microorganisms refer to the existence of human and/or animal faecal sources.

In field studies with injection tracers and microorganisms, a limited spectrum of tracers and microorganisms is used. Solute tracers most used are sodium chloride and sodium bromide, and some dyes, which are expected to behave conservatively, *i. e.* have no interaction with the porous medium, and therefore, provide information on dilution, water flow velocities and dispersion. Microorganisms most used for injection are those that resemble pathogenic microorganisms in the properties determining their removal (attachment, inactivation/die-off). Moreover, model organisms are used that behave relatively conservative (poor attachment and high persistence), because of the wide variability in those properties amongst the pathogens that they represent. For viruses, bacteriophages are used and for bacteria *E. coli* is the common choice. In the case of monitoring naturally present microorganisms, so-called indicator organisms, such as bacteriophages and fecal coliforms or *E. coli* are used. The indicator function refers to a similar behaviour as the pathogen. In the case of injection studies particular bacteriophages or strains of *E. coli* are used. Naturally occurring faecal indicator organisms are always groups bacteriophages, usually somatic coliphages or F-specific RNA bacteriophages, and various wild type coliforms. Indicator organisms are commonly present in concentrations that are several orders in magnitude higher than the concentrations of their pathogenic counterparts, and, therefore, are easier to detect. Moreover, excretion of faecal indicator organisms is continuous, whereas that pathogens requires infection.

3.2 Microbial Indicators/ Models

3.2.1 Bacteriophages as model viruses

Viruses vary greatly in their ability to travel through the subsurface. Research on groundwater protection against virus contamination is therefore restricted to using model viruses that are on the conservative side of the spectrum, implying little interaction with the soil and high persistence in water. If groundwater is adequately protected against contamination with such model viruses, one can be confident about adequate protection against contamination with all waterborne pathogenic microorganisms. Suitable model viruses to study virus transport are poorly attaching and stable bacteriophages such as PRD1. Bacteriophages are harmless viruses because they cannot infect a person; instead, they need a specific bacterial cell for multiplication. Bacteriophages are also easy to count in the laboratory (Schijven and Hassanizadeh, 2000). Information on bacteriophages and their use as general and host-associated faecal indicators and the application of phages as treatment indicators can be found in the chapter, General and host-associated bacteriophage indicators of fecal pollution.

F-specific bacteriophages have similar properties as enteroviruses, especially regarding size, shape and surface charge (Schijven and Hassanizadeh, 2000). Moreover, like enteroviruses, they are naturally present in the aquatic environment but in concentrations that are 100–10 000 times higher than concentrations of enteroviruses. Concentrations of enteroviruses in raw wastewater may be on the order of a few hundred virus particles per liter and in river water, under the influence of wastewater discharges, on the order of 0.01 to 1 virus particles per liter

(Lodder et al., 2005). Schijven and Hassanizadeh (2000) reasoned that, in the environment, enteroviruses and F-specific bacteriophages follow largely the same water pathways. Both have passed the sewerage system, were discharged into surface water and may have entered groundwater, for example by river bank filtration. Along these pathways, the less persistent viruses and those that attach readily to solid surfaces will have disappeared. This implies that selection of the most persistent and the least attaching viruses takes place amongst enteroviruses as well as bacteriophages. The naturally higher concentrations of bacteriophages allows determining virus removal by soil passage of four to about eight $\log_{10}$. For more information on this type of phage see also General and host-associated bacteriophage indicators of fecal pollution.

A large body of column and field studies exist in which bacteriophage MS2 was used as a model virus for studying virus transport in the subsurface. Bacteriophage MS2 is a group I F-specific bacteriophage. It is icosahedral with a diameter of 26 nm and has a low isoelectric point of 3.5, implying it has a negative surface charge under most conditions. As Schijven and Hassanizadeh (2000) have reviewed, in most soils it therefore attaches less than or as poorly as other negatively charged viruses. For example, a coxsackievirus B4 attaches as poorly as MS2, whereas, the less negatively charged, poliovirus 1 attaches much more (Schijven et al., 2003). At low temperatures, it is relatively persistent, but much less so at temperatures above 10 °C. It is easy to enumerate by double agar layer technique using *E. coli* or *Salmonella typhimurium* strains that can form F-pili to which MS2 may attach. The plaques that form in the agar plates are small and can be faint. Plaque-forming efficiency depends on the bacterial host and may vary between laboratories. Details on MS2 bacteriophage can be found on chapter 13.

Harvey and Ryan (2004) and Schijven and Hassanizadeh (2000) reviewed literature on the use of bacteriophage PRD1 as a model virus. It is an icosahedral bacteriophage with a diameter of 62 nm. It attaches about as poorly as MS2. It has the advantage over MS2 that it is much more persistent, in fact more than most viruses (Bertrand et al., 2012). The combination of these two properties makes it an excellent precautious model virus. In addition, it is easier to enumerate PRD1 than MS2. PRD1 plaques are bigger and clear and there is less variability in plaque-forming efficiency because it is a somatic bacteriophage. PRD1 has structural and functional similarities with mammalian adenoviruses.

3.2.2 *E. coli* as model bacterium

E. coli and otherwise faecal coliforms are the most used indicators of faecal contamination of water (WHO, 2011). Detailed information on *E. coli* as faecal indicator can be found in chapter 12. Chapter 16 gives information on *E. coli* as treatment indicator. Because of this indicator role, it is by default a microbial tracer for transport of bacteria transport in the subsurface. The following information on *E. coli* in that role was taken from the review of Foppen and Schijven (2006). *E. coli* is a more hydrophilic than hydrophobic microorganism, and, consequently, its hydrophobicity does not determine attachment. Instead, the major factor determining attachment of *E. coli* is its surface charge. In solutions with mono- and bi-valent electrolytes at 10^{-5} to 400 mM and pH 7.2–8.8 zetapotentials (net surface charge) range from -20 to -170 mV. In the presence of bivalent ions, the zetapotential is least negative and rather insensitive to pH. Zetapotential depend very much on the growth phase of *E. coli* and varies widely between different strains. In general, because of their net negative charge, and therefore little attachment (Foppen and Schijven, 2006) and their persistence in the environment (Franz et al., 2014), they may travel far in the subsurface. Using data from field and laboratory studies, Foppen and Schijven (2006) estimated that sticking efficiencies vary between 0.002 and 0.2 for a range of geochemically heterogeneous sediments under various hydrochemical conditions. Sticking efficiencies are a measure of attachment. It is the fraction of particles (in this case *E. coli* cells) that remain attached (stick to) the solid surface of grains particles after collision with thesoil grains.

3.2.3 Bacterial spores as model protozoa

A number of studies exist on the transport of anaerobic and aerobic spores in porous media, as potential surrogate for *Cryptosporidium* oocysts. The latter are known to be very persistent, but the persistence of bacterial spores widely exceeds that of oocysts. Also oocysts are about five times larger in diameter than bacterial spores. Further information to use bacterial spores as treatment indicators is given in chapter 16.

Schijven et al. (2000) found that *Clostridium bifermentans* R5 spores were removed by attachment by about 5 $\log_{10}$ in the oxic zone of a deep sandy aquifer, but was transported almost conservatively in the anoxic zone. Schijven et al. (2003) found that removal of *Clostridium perfringens* D10 spores by passing through a sand column was difficult to determine. These spores attached readily, but detached readily as well. Because inactivation of bacterial spores is negligible, and if there is no other irreversible removal process, all spores break through eventually. Hijnen et al. (2004) found that spores of sulphite reducing clostridia (anaerobic spores) were not a good quantitative surrogate for protozoan oocyst removal by slow sand filtration, simply because the latter are removed much more.

3.3 Synthetic Surrogates

3.3.1 Unmodified Microspheres

Synthetic fluorescent microspheres have been often used as safe surrogates to gain information about the abiotic aspects of microbial transport behavior in groundwater (Harvey et al., 2011a, 2011b; Harvey and Harms, 2002). Synthetic microspheres are biologically inert, chemically stable and commercially available in various sizes with well-defined properties. In most transport studies, carboxylated fluorescent polystyrene microspheres are used as surrogates as they are negatively

charged like microorganisms. Fluorescent polystyrene microspheres proved to be non-genotoxic and are safe tracers from toxicological consideration (Behrens et al., 2001). Fluorescent microspheres can be detected by using epifluorescence microscopy, flow cytometry, solid-phase cytometry, spectrophotometry and spectrofluorimetry. For simplicity, we will use “microspheres” for all synthetic beads regardless of their sizes.

3.3.1.1 Microspheres as virus surrogates

Most existing transport studies were limited to comparisons of microspheres and bacteriophages of similar sizes, i.e. surrogate-surrogate comparisons. Significant differences were found between microspheres and bacteriophages of similar sizes in their attenuation and transport behaviors (Mondal and Sleep, 2013; Weisbrod et al., 2013; Bales et al., 1997). Compared to the phages, microspheres either showed significantly greater removal (Weisbrod et al., 2013; Mondal and Sleep, 2013) or significantly less removal (Bales et al., 1997). The only published microsphere - virus pathogen comparison was given in Stevenson et al. (2015). In their column study, 100 nm carboxylated polystyrene microspheres showed 5.2–7.8 $\log_{10}$ removal but human adenovirus showed only 3.3–4.2 $\log_{10}$ removal, implying that the unmodified microspheres over-predicted adenovirus removal by a few orders of magnitude.

3.3.1.2 Microspheres as bacteria surrogates

The existing studies focused on comparisons of bacterium-sized microspheres (0.2–1.0 µm in size) with indigenous bacteria of groundwater. Little information is available in the comparisons of bacterium-sized microspheres with pathogenic bacteria. Bacteria-sized microspheres are considered relatively poor surrogates in predicting bacterial transport within porous granular media (Harvey et al., 1989; Harvey and Garabedian, 1991). In the review of Harvey et al. (2011), it is indicated that although retardation of microspheres was reasonably close to that of the bacteria, fractional loss of the microspheres was generally much greater.

3.3.1.3 Microspheres as protozoan surrogates

Many laboratory studies have compared the attenuation and transport of *Cryptosporidium parvum* oocysts and oocyst-sized carboxylated polystyrene microspheres (3–5 µm) in porous media. Comparisons can be found in the reviews of Harvey et al. (2011a, 2011b), and in other studies (Tufenkji and Elimelech, 2005; Mohanram et al., 2010; Pang et al., 2012; Stevenson et al., 2015a). Most transport studies with sand media employed flow rates of less than 2 m/day, simulating field groundwater velocity in sand aquifers. Substantial differences were observed in most transport studies between microspheres and oocysts in their attachment and transport behaviors (Bradford and Bettahar, 2005; Tufenkji and Elimelech, 2005; Harvey et al., 2008; Metge et al., 2010; Pang et al., 2012; Stevenson et al., 2015a). In comparison with oocysts, microspheres showed substantively less removal (Tufenkji and Elimelech, 2005; Metge et al., 2010; Pang et al., 2012; Stevenson et al., 2015a).

3.3.2 Biomolecule-modified microspheres

As described above, unmodified microspheres do not mimic well the attenuation and transport behaviors of target microorganisms in most transport studies. This is largely because unmodified microspheres are very different from the target microorganisms in their surface characteristics (e.g. surface charge, surface structure, hydrophobicity etc.). For example, unlike oocysts, unmodified microspheres do not have surface macromolecules. Likewise unlike viruses, unmodified microspheres do not have protein capsids. As surface properties play a very important role on particle retention and transport in porous media, Pang et al. (2012; 2014) have developed a new approach in using biomolecule-modified microspheres to mimic the physiochemical properties and surface characteristics of a target pathogen. They hypothesize that the retention and transport of a persistent pathogen in porous media could be mimicked using biomolecule-modified particles that are of similar size, surface charge, density, shape to the target pathogen and like pathogens they could have surface macromolecules such as protein coats. These properties have a significant influence on pathogen retention and transport in porous media. Using this concept, new surrogates for *Cryptosporidium parvum* (Pang et al., 2012) rotavirus and adenovirus (Pang et al., 2014) have been developed (Figure 3, Table 8). These are described below.

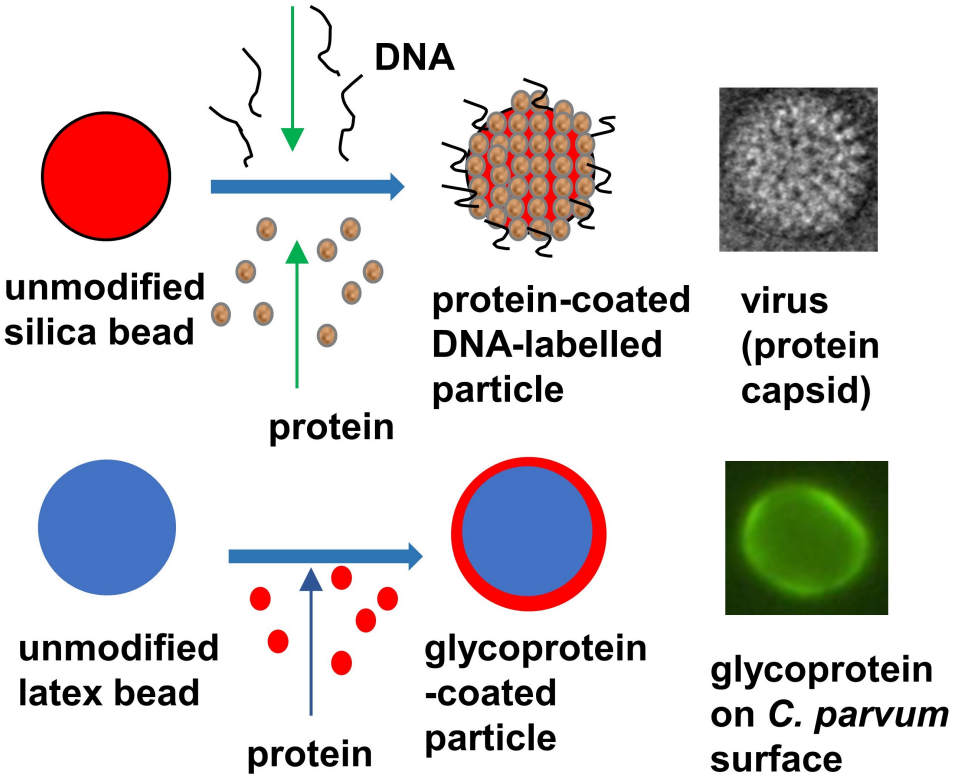

Figure 3. Illustration of micro mimics – surface modification (based on Pang et al., 2012 and 2014).

Table 8. Similar properties of Cryptosporidium and the surrogate

Properties	*Cryptosporidium*	Surrogate
Average size (µm)	4.9 (± 1.0)	4.9 (± 0.1)
Buoyant density (g/cm^3)	1.05	1.05
Shape	Spherical/oval	Spherical
Signal for detection	Fluorescently stained	Fluorescent beads or DNA labelled
Surface charge (mV)	-12 (± 0.5)	-11 to -18
Surface macromolecules	Glycoprotein	Glycoprotein

Summarized from: Pang et al., 2012

3.3.2.1 Rotavirus and adenovirus surrogates

The virus surrogates were synthesized by covalently coating the selected proteins and a double-stranded 302 base-pair synthetic DNA (link to glossary) onto 70 nm carboxylated silica beads. The selected proteins and DNA marker had similar surface charges to those of the target viruses. Three types of rotavirus surrogates (Figure 4) were produced with glycoprotein (type 1), protein A (type 2) and α_1-microglobulin/bikunin precursor (type 3), respectively. The adenovirus surrogate was simply DNA-labelled silica beads. Filtration experiments with beach sand demonstrated the similarity of the virus surrogates' concentrations, filtration efficiencies and attachment kinetics to those of the target viruses (Pang et al., 2014). The surrogates showed the same magnitude of concentration reduction as the viruses (Figure 4). Conversely, MS2 phage over-predicted concentrations of adenovirus and rotavirus by 1- and 2-orders of magnitude respectively. The new surrogates remained stable in size,

surface charge and DNA concentration for at least one year. Preliminary tests suggest that DNA-labelled microspheres were readily detectable in a number of environmental waters and treated effluent. PRD1 was found to be the most appropriate surrogate for adenovirus in an aquifer dominated by calcite material but not under high ionic strength or high pH conditions (Stevenson et al., 2015b).

Hydrophobicity of rotavirus, rotavirus surrogates, and MS2 bacteriophage was investigated by Farkas et al. (2014). Compared to MS2, the surrogates generally better mimicked rotavirus hydrophobicity and rotavirus adsorption to unmodified and hydrophobic sand. In contrast, MS2 was significantly more hydrophobic than rotavirus and the rotavirus surrogates, and MS2 showed remarkably greater adsorption to the hydrophobic sand. Burbery et al. (2015) have also found MS2 strongly attaches to plastic (hydrophobic in nature) apparatus in column experiments. This suggests that, when hydrophobic material (e.g. organic matters) is present, MS2 is not a conservative surrogate and will over-predict virus removal. This would have some important implications in virus transport in water repellent (hydrophobic) soils.

3.3.2.2 *Cryptosporidium parvum* surrogates

C. parvum surrogates were synthesized by covalently coating glycoprotein or biotin onto carboxylated fluorescent polystyrene microspheres that have size, density, and shape similar to *C. parvum* (Table 8). These biomolecules have isoelectric points similar to that of *C. parvum* (pH ≈ 2) and glycoprotein is a major type of surface protein that oocysts possess. In the column filtration experiments with alluvial sands, the surrogates showed the same log reduction in concentration as oocysts, whereas results from unmodified microspheres deviated by 1-order of magnitude (Figure 4). Compared to biotin-coated microspheres, glycoprotein-coated microspheres better resembled oocyst concentrations and attachment-detachment pattern. The surrogates remained stable in size and surface charge for at least 22 months. These new surrogates were further validated by Stevenson et al. (2015) in filtration experiments with granular limestone aquifer media and similar findings were reported.

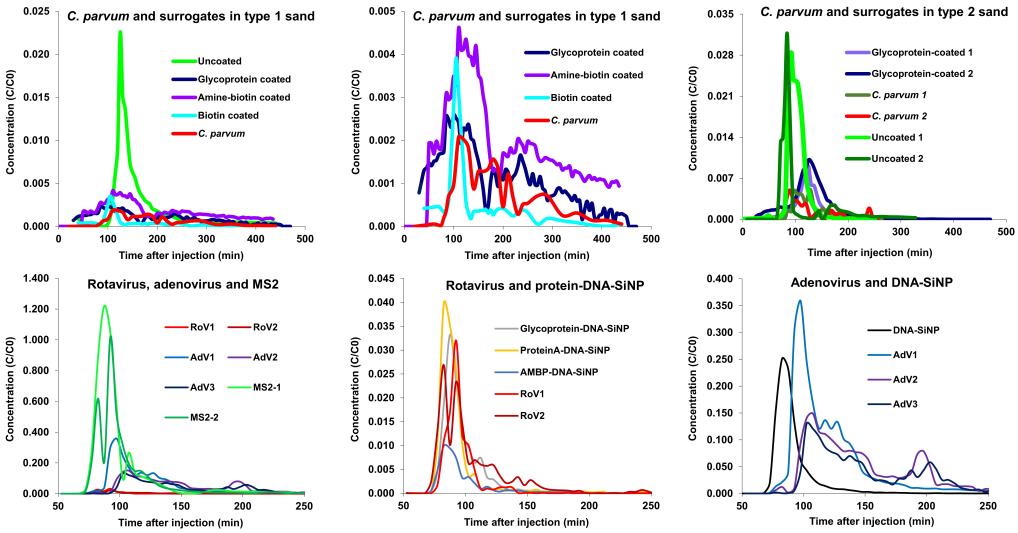

Figure 4. Micro mimics – mimicking pathogen filtration removal in aquifer media (modified after Pang et al., 2012; modified from Pang et al., 2014 with authors' additional data)

3.4 Flow Tracers (Salts, Dyes and Synthetic DNAs)

Chemical solute tracers are widely used to determine hydraulic connection, velocity and dispersion of groundwater and soil-water (Table 1). The most commonly used solute tracers are chloride (Cl^-), bromide (Br^-), and fluorescent dyes although other less used solute tracers also exist (e.g. nitrate, stable isotopes etc.). In the last 16 years, synthetic DNA tracers have been also developed for water tracing purposes. Cl and Br are chemically conservative or nonreactive in most subsurface media while dye tracers and DNA traces are less conservative.

3.4.1 Chloride

Chloride is a safe tracer for the environment as it is not associated with any direct health effects. No health-based guideline value is proposed for chloride in drinking-water (WHO, 2011). Due to its high natural background, large injection volume at very high chloride concentrations is needed in salt tracer experiments. The injection of high chloride concentration can cause strong density effects thereby jeopardizing the usefulness of the recorded breakthrough curves (Schmid et al., 2004). In addition, the spatial background levels of chloride are often variable, which could increase the uncertainty of tracer results.

3.4.2 Bromide

Aiming to protect aquatic organisms that have the lowest tolerance to Br from chronic toxicity, a water quality criterion of 1 mg/L bromide has been proposed (Canton et

al., 1983; Flury and Papritz, 1993). When using Br for water tracer experiments, Br- concentrations in downstream receiving waters need to be carefully evaluated. If the receiving water is used as the source water for drinking water supplies, the disinfection (chlorination and ozonation) of bromide-containing source water can result in a formation of carcinogenic brominated organics (disinfection by-products) and bromate (WHO, 2011).

Bromide is a frequently used conservative tracer in laboratory and field studies. Bromide ions have physical and chemical properties very similar to chloride ions but are at much lower natural background levels. Bominde has a low toxicity in mammals, most freshwater organisms and most plants (Flury and Papritz, 1993).

3.4.3 Fluorescent dyes

Fluorescent dyes are effective tracers for determining and visualizing flow direction and pathways, and to large extent, flow velocity and dispersion in subsurface media. Fluorescent dyes can be visually detected at ppb levels (Smart et al., 1998) thus they are often used for qualitative determination of flow paths. When quantitative flow data are required, fluorescent dyes can be measured easily and inexpensively by filter fluorometers and spectrofluorometers. Being relatively large organic molecules, fluorescent dyes are more or less absorbed in subsurface media but most synthetic dyes are resistant to degradation in the environment (Flury and Wai, 2003). Fluorescent dye tracers have low to moderate levels of concern of toxicity to human health and aquatic organisms (Field, 1995). Of all the available dye tracers, Fluorescein (uranine) and Rhodamine WT are the most used in tracing studies (Flury and Wai, 2003). Fluorescein is considered to be non-carcinogenic (Smart, 1984; Behrens et al., 2001) but there are conflicting reports on rhodamine WT (Smart and Laidlaw, 1977; Smart, 1984; Behrens et al., 2001). Brilliant blue FCF is considered to be non-carcinogenic (Flury and Flu¨hler, 1994). WHO Guidelines do not refer to Fluorescein and Rhodamine dyes. The standards established by USEPA in the Federal Register (Vol. 63, No. 40) state the maximum Rhodamine WT concentrations to be 0.1 ppb in drinking water, 10 ppb for water entering a drinking water plant (prior to treatment and distribution) and 100 ppb for groundwater not associated with drinking water production (USEPA, 1998).

3.4.4 Synthetic DNA tracers

Synthetic DNA tracers, with their unique fingerprints can provide highly specific information in contamination pathways. Synthetic DNA has no background in the environment and can be detected qualitatively by PCR (polymerase chain reaction) and quantitatively by qPCR at very low concentrations. Unlimited number of DNA tracers can be designed using random sequence generator software. The use of multiple DNA tracers allows simultaneously identifying and characterizing different contamination sources and pathways (Sabir et al., 2001; Ptak et al., 2004). Since synthetic DNA are not derived from the genome of any organism, they do not have genetic functionality thus are environmentally safe (Sharma et al., 2012). DNA tracers have been successfully applied in field tracing studies in fractured rock aquifers (Colleuille and Kitterod, 1998; Sabir et al., 2000), alluvial sand and gravel aquifers (Sabir et al., 1999; 2000; Ptak et al., 2004), karst groundwater systems (Aquilanti et al., 2013; Bovolin et al., 2014) and surface streams (Foppen et al., 2011; Foppen et al., 2013). However DNA tracers are susceptible to microbial activity, light, temperature and chemicals and can adsorb to subsurface media (Ptak et al., 2004). Thus DNA tracers are more suitable in tacking fast-flow groundwater in karst systems (Aquilanti et al., 2013; Bovolin et al., 2014), fractured rocks and sandy gravel aquifers (Sabir et al., 2000).

3.5 Emerging Chemical Sewage Markers

Pharmaceuticals and personal care products (PPCPs) have been used as emerging chemical sewage indicators and/or markers of domestic sewage pollution in soils and groundwater (Table 1). Detection of various human PPCPs in soils and groundwater could indicate water and soil contamination with human sewage as these chemicals are consumed and/or excreted by humans. Some organic compounds associated with human activities such as sterols (5β-coprostanol), linear alkylbenzenes, trialkylamines, fluorescent whitening agents, carbamazepine, triclosan, as well as caffeine and nicotine derivatives have been proposed as prospective anthropogenic markers of sewage contamination (Chen et al., 2014; Kasprzyk-Hordern et al., 2009; Oppenheimer et al., 2012; Seiler et al., 1999; Wolf et al., 2012). Some commonly used chemical tracers are introduced as follows.

3.5.1 Caffeine

Caffeine is present in many products consumed daily such as coffee, tea, soft drinks, and chocolate. The average consumption of caffeine is ~131 mg/person/day in the USA and 190–410 mg/person/day in Australia (Froehner et al., 2010). Caffeine has a high solubility in water (13 g/L), a low octanol–water coefficient (log K_{ow} 0.01), and insignificant volatility. Thus it fits the profile for a good, stable, dissolved marker for anthropogenic influences. In a contaminated river, caffeine showed a positive correlation with coprostanol, a faecal biomarker (Froehner et al., 2010). Caffeine is a ubiquitous compound in raw domestic wastewater with typical loads of approximately 16 mg/person/day (Buerge et al., 2006). In wastewater treatment plants (WWTPs) of the Freifensee region, caffeine is largely eliminated (>99%), resulting in much smaller loads of <0.15 mg/person/day in treated wastewater. Therefore, caffeine is a suitable marker for untreated wastewater. Caffeine was found at concentrations up to 0.23 µg/L in some shallow wells of Nevada, US along with elevated nitrate concentrations, but not detected in deep wells (>10m), providing a clear evidence of domestic wastewater contamination in the study area (Seiler et al., 1999). It should be pointed out that caffeine is not a conservative marker, so it is better used in combination with conservative markers such as

carbamazepine in order to trace sewage contamination sources.

3.5.2 Artificial sweeteners

Artificial sweeteners are used as substitutes for sugar in food additives, health and dietary products (foods and beverages) and animal feeds. Artificial sweeteners are excreted mostly unchanged from human body and flow down the drain into wastewater treatment plants. They are hydrophilic compounds with solubility of 565–9.1×10^5 at 25ºC (Subedi and Kannan, 2014). There are large variations in their removal efficiencies in WWTPs. Both saccharin and aspartame were reported to be significantly removed (>68%) in WWTPs, while sucralose and acesulfame were barely removed (Buerge et al., 2009; Gan et al., 2013; Scheurer et al., 2009; Subedi and Kannan, 2014). Artificial sweeteners degrade at varying rates under different environmental conditions. Incubated in aerobic soils for a period of 1–3 months, acesulfame and sucralose showed very slow microbial degradation (Buerge et al., 2010). Therefore, sucralose and acesulfame could be good chemical markers and tracers of wastewater contamination in groundwater. Acesulfame was detected in groundwater samples of a regular monitoring program in Zurich, Switzerland with concentrations up to 4.7 µg/L (Buerge et al., 2009). There is a link between the high concentrations in groundwater and upstream infiltrating river water as found in the study. The presence of acesulfame in groundwater may thus indicate infiltration of treated domestic wastewater via surface water (indirect inputs) but may also indicate untreated wastewater, e.g., from a leaky sewer (direct inputs). Because of its persistence, acesulfame does not discriminate between treated and untreated wastewater. However, the absence of saccharin and cyclamate in the investigated groundwater samples suggests that contamination primarily originated from treated wastewater.

3.5.3 Carbamazepine

Carbamazepine is an important drug for the treatment of epilepsy, as well as various psychotherapy applications. It has a high water solubility and a log Kow of 2.25. Studies have showed that carbamazepine is one of the most frequently detected pharmaceuticals in wastewater treatment plants and receiving waters and it has low removal rates (<30%) in WWTPs, and majority exists in the aqueous phase. Thus it has been widely used as a marker of domestic sewage inputs (Glassmeyer et al., 2005; Kasprzyk-Hordern et al., 2009; Madoux-Humery et al., 2013; Miao et al., 2005; Ternes TA, 1998). For example, carbamazepine was detected in groundwater in Canada (Van Stempvoor et al., 2013) and Germany (Wolf et al., 2012). The co-tracers (acesulfame, sulfamethoxazole and carbamazepine) were successfully applied to infer the presence of municipal waste water plumes.

3.5.4 Iodinated X-ray contrast media

Iodinated X-ray contrast media (ICM) are frequently applied in clinical diagnosis for the purpose of imaging soft tissues like blood vessels, organs and lacunae. ICM are highly water solubility. Given their very low biodegradability and no background environmental concentration, ICMs have been used as wastewater markers. The ICM amidotrizoic acid, the anticonvulsant carbamazepine and artificial sweetener acesulfame were detected in about 30% of urban groundwater due to leaking sewer networks in Rastatt city, Germany (Wolf et al., 2012). The spatial distribution of chemical markers corresponds well as with predictions by pipeline leakage models.

4.0 Modelling Fate and Transport of Microorganism in the Subsurface

4.1 Governing Model Equations

The processes that govern the fate and transport of microorganisms in the subsurface include advection (the microorganisms are transported by the flowing water), dispersion (during transport not every microorganism follows the same pathway), persistence (microorganisms disappear from the system because they die-off (bacteria, protozoa) or are inactivated (viruses); the most persistent ones, survive the longest), attachment (microorganisms stick to the solid surface of the soil grains), detachment (attached microorganisms are remobilised and re-enter the water phase) and straining (the microorganisms are too large to pass a pore throat of the porous medium). In this section, the principles of these processes are explained. The governing mathematical equations are given and it is explained how the transport processes can be identified and quantified (values of model parameters) from breakthrough curves (column and field studies) and batch experiments (microorganisms suspended in water).

A complete advection-dispersion equation of microorganism fate and transport, including exchange to the solid surfaces of the soil grains (attachment and detachment) as well as to the air-water interface (AWI; in the case of unsaturated conditions), in one-dimensional form, is given by Bradford et al. (2013; 2014):

$$\frac{\partial \theta_c C}{\partial t} + \rho_b \frac{\partial S}{\partial t} + \frac{\partial A_{aw}\Gamma}{\partial t} = \frac{\partial}{\partial t}\left(\theta_c D \frac{\partial C}{\partial z}\right) - \frac{\partial q_c C}{\partial z} + B_w \qquad (2)$$

where C [L^{-3}] is the microorganism concentration in the water, S [M^{-1}] is the microorganism concentration retained on the solid-water interface (SWI), G [L^{-2}] is the microorganism concentration retained on the AWI, θ[L^3L^{-3}] is the volumetric water content accessible to the microorganisms, D [L^2T^{-1}] is the hydrodynamic dispersion coefficient for microorganisms, r_b [ML^{-3}] is the bulk density, A_{aw} [L^2L^{-3}] is the air-water interfacial area per unit volume, q_c [LT^{-1}] is the volumetric water flux density for colloids, B_w [$L^{-3}T^{-1}$] represents inactivation or die-off of the microorganisms in the water (in this context growth is not considered), z [L] is the distance in the transport direction, and t [T] is the time. The first term on the right side of equation (1) represents the dispersive and advective fluxes

of the microorganisms, respectively. The second and the third terms on the left side of equation (1) represent the mass-transfer terms from the water to the SWI and AWI in units of $L^{-3}T^{-1}$. Due to ion or size exclusion, q_c may be smaller than the total volumetric water content q_w [-].

As Bradford et al. (2013) mention, exchange of microorganisms with the SWI and AWI can be modelled as equilibrium or kinetic processes that are reversible or irreversible. For microorganisms, generally, equilibrium processes are not considered (Schijven and Hassanizadeh, 2000). Commonly, attachment has been assumed to be the most dominant mechanism of microorganism retention in porous media. The following equations describe mass-transfer of microorganisms between the SWI and AWI because of attachment and detachment (Bradford et al., 2013; Simunek et al., 2006):

$$\rho_b \frac{\partial S_a}{\partial t} = f_{sw}\theta_c \Psi_{sw} k_{asw} C - \rho_b k_{dsw} S_a \qquad (3)$$

$$\frac{\partial A_{aw}\Gamma}{\partial t} = f_{aw}\theta_c \Psi_{aw} k_{aaw} C - \mathrm{A}_{aw} k_{daw} \Gamma_a \qquad (4)$$

where k_{asw} [T^{-1}] is the first-order attachment coefficient to the SWI and k_{dsw} [T^{-1}] is the first-order detachment coefficient from the SWI. Likewise k_{aaw} [T^{-1}] is the first-order attachment coefficient to the AWI and k_{daw} [T^{-1}] is the first-order detachment coefficient from the AWI. , Y_{sw} [-] and Y_{aw} [-] are retention functions that account for blocking or ripening. Only a fraction of the SWI (f_{sw}) and AWI (f_{aw}) may be accessible for attachment due to size exclusion of microorganisms from small pore spaces or thin water films. Attachment/detachment to the SWI may also be needed to model using two types of sites on the SWI (S_1 and S_2) (Schijven et al., 2002). The number of sites for attachment may be limited. Especially under unfavourable conditions for attachment, this may be the case (Bradford et al., 2014). According to the Langmuir model:

$$\Psi_{sw} = 1 - \frac{S_a}{S_{max}} \qquad (5)$$

Where S_{max} [M^{-1}] is the maximum concentration of attached microorganisms. This implies that the attachment rate declines with time as sites for attachment become filled. Such a condition may occur near a site where wastewater enters groundwater and where there is a large mass of solid particles competing for the same attachment sites.

Bradford et al. (2014) proposed to use a two region model to simulate different rates of microbial transport that occur in the bulk aqueous phase and in low-velocity regions. This model is equivalent to the dual-permeability transport model and is suited to handle the distinction between the presence of both favourable and unfavourable conditions for attachment that are frequently the case under natural conditions.

4.2 Attachment: Colloid Filtration Theory

The attachment of a microorganism (or in general: a colloid) to the SWI involves two processes: first, mass transport to the surface and then interaction between the surface of the microorganism and the solid surface. This means that k_{asw} depends on microscale flow and diffusion characteristics as well as surface properties of the microorganism and the soil grains. These process are described by colloid filtration theory (CFT), which allows exclusion of the effects of flow and diffusion by expressing the attachment rate of microorganisms in terms of single-collector contact efficiency) h [-] and sticking efficiency a [-]. According to CFT, a suspended particle may come into contact with a solid surface (the collector) either by interception (the collector is on the flow path of the particle), sedimentation (the particles settles on a surface because of gravity, of relevance for bacteria and protozoa), or diffusion (Brownian motion, typical for small particles, like viruses) (Yao et al., 1971):

$$k_{asw} = \frac{3}{2}\frac{1-\theta}{d_{50}}\alpha\eta\nu \qquad (6)$$

where d_{50} [L] is the median soil grain diameter, q [L^3L^{-3}] is the volumetric water content and v is average pore water velocity. Note that η and α are probabilities and, by definition, their values must lie in the range of zero to one. Single-collector contact efficiency η can be predicted from the physical characteristics of the domain. In laboratory and field experiments, the value of k_{asw} may be determined. Commonly, the value of the sticking efficiency α is then derived from η and k_{asw}. In doing so, at times, the estimate of α may turn out to be larger than one. This indicates that another process is also removing microorganisms from the water phase, or the single-collector contact efficiency is underestimated. Sticking efficiency α depends on the surface properties of the microorganism and the soil grains. Tufenkji and Elimelech (2004) provide a correlation equation for predicting the single-collector contact efficiency in physicochemical particle filtration in saturated porous media. The correlation equation assuming that the overall single-collector efficiency is the sum of the Brownian diffusion, interception, and gravitational sedimentation. Viruses are small and Brownian diffusion determines their contact efficiency. Compared to other microorganisms, η of viruses is high. Also η of protozoa is relatively high, mainly due to sedimentation. In the case of bacteria of about 1 µm in size, η is at its lowest value. Alternative correlation equations are given by *e.g.* Rajagopalan and Tien (1976), Nelson and Ginn (2006), Ma et al. (2009) and Seetha et al. (2015).

The sticking efficiency represents the net effect of repulsive and attractive forces between the surfaces of the microorganism and soil grains. Therefore, it depends on pH, organic carbon content, and ionic strength.

4.3 Interaction Between the Surfaces of Microorganisms and Soil Grains

At the micro-scale, transport processes are viewed within a single pore between sand grains (porous medium).

Attachment of microorganisms to soil grains is primarily controlled by the interactions that act between the surfaces of the microorganisms and the soil grains at less than ten nanometre distance. These interactions encompass hydraulic interactions and electrostatic interactions, van der Waals attraction and Born repulsion. The latter three are described by DLVO theory (e.g. Tufenkji and Elimelech, 2004), which is a useful theoretical paradigm. Viruses consist of nucleic acid enveloped in a protein-coat. Like all proteins, a virus particle has an amphoteric nature, i.e. the carboxyl and amino groups of the amino acids determine its electric charge, which mainly depends on pH. Similarly, bacteria and protozoa have cell walls of amphoteric nature, moreover, they include a complexity of macromolecules.

Most poorly attaching microorganisms have a low isoelectric point, which is the pH where the net electric charge is zero. In groundwater, pH is usually 7–8, where most microorganisms have a net negative charge. At this pH, quartz surfaces (sand) also are negatively charged and, therefore, repel negatively charged microorganisms. This is the so-called unfavourable condition for attachment. Sand grain surfaces may contain patches of metal oxides that are positively charged, which are attractive for virus particles and, therefore, form favourable sites for attachment. The attractive and repulsive forces between surfaces of virus particles and the sand grains can be used to construct a DLVO energy profile. Favourable conditions for attachment occur when there is electrostatic attraction, i.e. the surface of the microorganisms and soil grains have opposite electric charges. In that case sticking efficiency α=1. In the case of unfavourable conditions, microorganisms and soil grains the same kind of (mostly negative) electric charge and repel each other and, therefore, α<1.

At least for bacteria and protozoa, but probably also, to some extent, for viruses (many viruses are icosahedral spheres, but also many are not), DLVO theory may not be adequate to describe the interactions between the surfaces of the microorganisms and soil grains. In their review, Ginn et al. (2002) mention that failures of DLVO theory, including extensions of DLVO theory for hydrophobic interactions, are most likely caused by the presence of polymers, other macromolecules, and structures such as pili and flagella on the bacterial surface. Such structures may cause steric repulsion or act as an attractive bridging force. In the presence of calcium ions, a discrepancy exists between DVLO theory and the experimentally determined double layer interactions between quartz grains and bacteriophage PRD1, possibly because the van der Waals interactions are underestimated or because of chemisorption of calcium ions on the surfaces (Sadeghi et al., 2013). Under unfavourable conditions, surface roughness, grain and colloid surface heterogeneity, and grain-grain contact points need to be considered as well (Seetha et al., 2015).

Note: In this chapter, the presented sticking efficiency values are derived from applying CFT (Tufenkji and Elimelech, 2004), *ie*. in laboratory and field experiments, values of the attachment rate coefficient, k_{asw}, were determined. Then, using CFT, values of the single-collector efficiency η were calculated, and, finally, by using equation (6), values for α were derived.

4.3.1 Blocking

Most colloid transport studies have considered clean bed conditions by assuming a constant attachment rate and infinite retention capacity. In reality, soil and aquifer material will always have a finite retention capacity and only a small fraction of the solid surface may contribute to colloid retention even under favourable attachment conditions (Sasidharan et al., 2014). Blocking of available retentions sites, like by other much more abundant particulate organic matter, decreases the attachment rate over time and enhances the transport of colloids. For predicting microorganism transport at field scale, blocking needs to be taken into account.

4.3.2 Straining

Microorganisms are strained in pore throats that are too small to allow passage. In homogeneous porous media, straining is not significant where the diameter of the microorganisms is less than 5% of the grain diameter, but in natural heterogeneous soils, a fraction of the pore diameters may be small enough (Ginn et al., 2002). Bradford et al. (2005) modelled straining as an irreversible first-order time and depth (travel distance) dependent process. Attachment and straining are not directly distinguishable from analysing breakthrough curves. Straining can be recognized from depth profiles of retained microorganisms that may be hyper-exponential when straining is involved.

4.3.3 Attachment/detachment from/to AWI

According to Bradford et al. (2014) there is no comprehensive theory to predict k_{aaw} and k_{daw} (equation 3). Adhesive interactions on the AWI are controlled by hydrophobic, electrostatic and capillary forces. Because the AWI is negatively charged at pH values found in most natural environment, the van der Waals interaction between microorganisms and the AWI is repulsive. Bradford et al. (2014) lists the literature on the increase of microbial retention under decreasing water saturation due to (1) sorption onto the AWI for positively charged or hydrophobic microorganisms, (2) physical restrictions imposed by thin water films (film straining), (3) retention at the air-water-solid contact line, (4) immobilization in dead-end pores, (5) enhanced deposition onto the SWI due to a reduction in the diffusive length in partially filled pores, and (6) a greater fraction of water flow in small pores spaces

Detachment of bacteria (*e.g.* Boks et al., 2008; Sharma et al., 2005) from solid surfaces has been studied by passing air bubbles in a parallel plate flow chamber. Detachment increased linearly with increasing air bubble velocity. Detachment by a passing air bubble depended greatly upon the bacterial strain, shape and size and solid surface. Apparently, attached colloids can detach very efficiently by means of moving AWIs. Torkzaban et al. (2006a; 2006b) found out that attachment and detachment

rate coefficients are different in columns with different water saturation. At the end of constant-saturation experiments, they drained or resaturated the column and observed that viruses attached to sand grains under saturated or unsaturated conditions could be remobilized by both drainage and re-saturation. This fast detachment was ascribed to physical forces induced by moving air-water interfaces AWIs, as was visualised with micromodels (Zhang et al., 2013). Zhang et al. (2012) developed a model to successfully simulate the transient effects on detachment of small colloidal particles under partially saturated flow on the basis of the data from Torkzaban et al. (2006a; 2006b). The model included the presence and variation of AWIs and assumed AWI attachment–detachment to be an equilibrium sorption process.

4.4 Inactivation

In equation (1) $B_w$represents inactivation or die-off of microorganisms, that occurs in the water, but also on the SWIs and AWIs. Commonly, in this area of research, inactivation or die-off are described as first-order processes. In many cases, especially short-term, this is appropriate. However, on the long term, it has been shown that viruses may inactivate initially faster than thereafter (de Roda Husman et al., 2009). In such cases, two-rate models or Weibull models (van Boekel, 2002) are needed to describe this non-linear behaviour. In first-order inactivation or die-off, the reciprocal value of the first order rate coefficient μ [day^{-1}] equals the T90 value, the time [day] after which 10% of the microorganisms persist. T90 is the times it takes by which numbers of microorganisms (or concentrations) have decreased a factor of ten (1 $\log_{10}$). For details on modelling inactivation or die-off and extensive persistence data, see Part Four. Risk and Management, Persistence and Transport.

Specific to groundwater a number of publications are available that provide persistence data and model parameter values. Clearly, environmental temperature is the dominant factor determining persistence. Bertrand et al. (2012) published a meta-analysis of virus inactivation and virus genome degradation data from literature. A linear model was employed by Bertrand et al. (2012) to analyse the effects of temperature, virus species, detection method (cell culture (CC) or molecular methods (PCR)), simple matrix (drinking water, groundwater, synthetic buffer) or complex matrix (seawater, freshwater, sewage, food, soil, biologic fluid and dairy products) and temperature range (<50°C and ≥50°C). Obviously, virus inactivation is much higher as temperatures increase (≥50°C), but there is also a significant temperature-matrix effect. Virus inactivation appeared to occur faster in complex matrices. Virus genome was shown to be more persistent than virus infectivity. Bertrand et al. (2012) provide estimates of the time (days) to the first $\log_{10}$ inactivation (TFL, the equivalent of often used T90 values), including uncertainty for a large number of viruses in different matrices. Table 9 lists TFL values for the different matrices and virus detection method (cell culture and PCR) for 5°C, 10°C, 15°C, 20°C, and 25°C. These lists provide a ranking of viruses are ranked to persistence and temperature sensitivity. These data also allow comparison between inactivation of infectious viruses (cell culture detection) and of infectious plus non-infectious viruses (PCR). Bacteriophage PRD1 appeared to be highly persistent under most conditions, which implies that it is a precautious indicator for virus inactivation studies. It should be noted that the 95%-prediction interval is wide (from approximately one-tenth to ten times the mean estimate). This is due the fact that many (unidentified) factors affecting virus inactivation underlie these data. Assuming first-order inactivation kinetics, the value of the inactivation rate coefficient can be calculated from TFL as follows:

$$\mu_l = ln10/TFL \qquad (7)$$

Table 9. T90 (Time in days to first log inactivation = reduction of concentrations by a factor of ten) of viruses

Matrix	Virus	5°C			10°C			15°C			20°C			25°C		
		EST[a]	95% PIb		EST	95% PI		EST	95% PI		EST	95% PI		EST	95% PI	
Complex matrix[c,d]	Bacteriodes fragilis phage	4.3	0.14	130	3	0.098	93	2.1	0.07	65	1.5	0.05	46	1.1	0.035	33
Complex matrix[c,d]	Coxsackievirus	14	1.2	170	10	0.83	120	7.1	0.59	85	5	0.42	60	3.5	0.29	43
Complex matrix[c,d]	Echovirus	6.2	0.5	75	4.4	0.36	53	3.1	0.26	37	2.2	0.18	26	1.5	0.13	19
Complex matrix[c,d]	FCV	5.8	0.47	70	4.1	0.34	49	2.9	0.24	35	2	0.17	25	1.4	0.12	17
Complex matrix[c,d]	FRNAPH all genogroups	14	1.1	180	10	0.79	130	7.1	0.56	89	5	0.4	63	3.5	0.28	45
Complex matrix[c,d]	FRNAPH genogroup I	14	1.2	170	10	0.86	120	7.1	0.61	82	5	0.43	58	3.5	0.31	41
Complex matrix[c,d]	FRNAPH genogroup II	11	0.82	150	7.9	0.58	110	5.6	0.41	76	4	0.29	54	2.8	0.21	38

Complex matrix[c,d]	FRNAPH genogroup III	11	0.82	150	7.9	0.58	110	5.6	0.41	76	4	0.29	54	2.8	0.21	38
Complex matrix[c,d]	FRNAPH genogroup IV	11	0.85	150	7.9	0.61	100	5.6	0.43	73	4	0.31	52	2.8	0.22	37
Complex matrix[c,d]	HAV	71	5.8	860	50	4.1	610	35	2.9	430	25	2.1	300	18	1.5	210
Complex matrix[c,d]	Human adenovirus	28	2.4	330	20	1.7	230	14	1.2	170	10	0.85	120	7.1	0.6	83
Complex matrix[c,d]	Human astrovirus	18	1.2	270	13	0.82	190	8.9	0.58	140	6.3	0.41	96	4.5	0.29	68
Complex matrix[c,d]	MNV	45	2.9	690	32	2.1	480	22	1.5	340	16	1	240	11	0.74	170
Complex matrix[c,d]	Poliovirus	18	1.5	210	13	1.1	150	8.9	0.77	100	6.3	0.54	73	4.5	0.38	52
Complex matrix[c,d]	PRD1 phase	71	6	830	50	4.3	580	35	3.1	410	25	2.2	290	18	1.6	200
Complex matrix[c,d]	Simian rotavirus	8.9	0.7	110	6.3	0.5	80	4.5	0.36	56	3.2	0.25	40	2.2	0.18	28
Complex matrix[c,d]	ϕX174 phage	89	7	1100	63	5	800	45	3.5	570	32	2.5	400	22	1.8	280
Complex matrix[c,e]	FCV	9.9	0.75	130	6.2	0.48	79	3.8	0.3	49	2.4	0.19	30	1.5	0.12	19
Complex matrix[c,e]	FRNAPH genogroup I	25	1.9	330	15	1.2	200	9.7	0.77	120	6	0.48	76	3.8	0.29	48
Complex matrix[c,e]	FRNAPH genogroup II	20	1.3	300	12	0.83	180	7.7	0.53	110	4.8	0.33	70	3	0.2	44
Complex matrix[c,e]	FRNAPH genogroup III	20	1.3	300	12	0.83	180	7.7	0.53	110	4.8	0.33	70	3	0.2	44
Complex matrix[c,e]	FRNAPH genogroup IV	20	1.3	290	12	0.86	180	7.7	0.55	110	4.8	0.34	67	3	0.21	42
Complex matrix[c,e]	HuNV	5.7	0.41	79	3.5	0.26	49	2.2	0.16	30	1.4	0.099	19	0.86	0.06	12
Simple Polymer Chain Reaction matrix[f,d]	CaCV	4.9	0.31	78	3.2	0.21	51	2.1	0.14	33	1.4	0.091	22	0.93	0.061	14
Simple Polymer Chain Reaction matrix[f,d]	Coxsackievirus	42	3	570	28	2	380	18	1.3	250	12	0.88	160	7.9	0.58	110
Simple Polymer Chain Reaction matrix[f,d]	Echorirus	17	1	270	11	0.69	170	7.2	0.46	110	4.8	0.3	76	3.2	0.2	50
Simple Polymer Chain Reaction matrix[f,d]	FCV	17	1	260	11	0.69	170	7.2	0.46	110	4.8	0.3	75	3.2	0.2	50
Simple Polymer Chain Reaction matrix[f,d]	FRNAPH genogroup I	42	3.2	550	28	2.1	360	18	1.4	240	12	0.92	160	7.9	0.61	100

Simple Polymer Chain Reaction matrix[f,d]	FRNAPH genogroup II	33	1	1100	22	0.68	710	14	0.45	470	9.5	0.3	310	6.3	0.2	200
Simple Polymer Chain Reaction matrix[f,d]	FRNAPH genogroup III	33	2.6	420	22	1.7	270	14	1.2	180	9.5	0.77	120	6.3	0.51	78
Simple Polymer Chain Reaction matrix[f,d]	FRNAPH genogroup IV	33	2.4	450	22	1.6	300	14	1.1	190	9.5	0.71	130	6.3	0.47	85
Simple Polymer Chain Reaction matrix[f,d]	HAV	210	17	2500	140	11	1700	91	7.6	1100	60	5	720	40	3.3	480
Simple Polymer Chain Reaction matrix[f,d]	Human adenovirus	83	6.8	1000	55	4.5	670	36	3	440	24	2	290	16	1.3	190
Simple Polymer Chain Reaction matrix[f,d]	Human astrovirus	52	4.3	640	35	2.9	420	23	1.9	280	15	1.3	180	10	0.82	120
Simple Polymer Chain Reaction matrix[f,d]	Human rotavirus	17	1.4	200	11	0.93	130	7.2	0.63	83	4.8	0.42	54	3.2	0.28	35
Simple Polymer Chain Reaction matrix[f,d]	MNV	130	8.6	2000	87	5.7	1300	58	3.8	870	38	2.5	570	25	1.7	380
Simple Polymer Chain Reaction matrix[f,d]	Poliovirus	52	4.5	610	35	3	400	23	2	260	15	1.3	170	10	0.87	120
Simple Polymer Chain Reaction matrix[f,d]	PRD1 phase	210	18	2400	140	12	1600	91	7.9	1100	60	5.2	700	40	3.4	460
Simple Polymer Chain Reaction matrix[f,d]	Simian rotavirus	26	2.2	320	17	1.4	210	11	0.96	140	7.6	0.63	91	5	0.42	60
Simple Polymer Chain Reaction matrix[f,d]	ϕX174 phage	260	21	3300	170	14	2200	110	9.1	1400	76	6	950	50	4	630

Simple Polymer Chain Reaction matrix[f,e]	CaCV	8.6	0.46	160	8.5	0.49	150	8.4	0.51	140	8.3	0.53	130	8.2	0.54	130
Simple Polymer Chain Reaction matrix[f,e]	Coxsackievirus	79	4.1	1500	78	4.3	1400	77	4.4	1300	76	4.4	1300	75	4.4	1300
Simple Polymer Chain Reaction matrix[f,e]	FCV	31	1.6	600	31	1.7	560	31	1.8	530	30	1.8	510	30	1.8	500
Simple Polymer Chain Reaction matrix[f,e]	HAV	79	4.1	1500	78	4.3	1400	77	4.4	1300	76	4.4	1300	75	4.4	1300
Simple Polymer Chain Reaction matrix[f,e]	HuNV	1.3	0.069	25	1.3	0.074	23	1.3	0.077	21	1.3	0.08	20	1.2	0.081	19
Simple Polymer Chain Reaction matrix[f,e]	Poliovirus	99	5.1	1900	98	5.4	1800	97	5.5	1700	95	5.6	1600	94	5.5	1600

Source: (Bertrand et al., 2012); [a]EST: Mean estimate; [b]PI: Prediction interval; [c]Complex matrix (seawater, freshwater, sewage, food, soil, biologic fluid and dairy products); [d]Cell culture; [e]PCR: Polymer Chain Reaction; [f]Simple Polymer Chain Reaction matrix (drinking water, groundwater, synthetic buffer).

By means of a meta-regression analysis, Franz et al. (2014) provided a quantitative summary of the variability in *E. coli* persistence in soil and water over a broad range of individual studies and to identify the most important sources of variability. Soil and water, the type of experiment (laboratory or field), the matrix subtype (type of water and soil), and temperature were the main factors determining survival. A higher average decline rate in soil of pathogenic *E. coli* than commensal *E. coli* was found. Pedley et al. (2006) provide tables with inactivation or die-off rate coefficients, from which T90 values can be derived easily, for viruses and (mostly indicator) bacteria in groundwaters. Foppen and Schijven (2006), also provide such values for *E.coli* in water.

Note that in the case of enumerating viable but nor culturable bacteria, or bacteria and viruses by means of quantitative PCR methods, inactivation or die-off are not considered. In a risk assessment context this may lead to an overestimation of risk. Lodder et al. (2013) compared breakthrough of bacteriophage MS2 through a slow sand filter by enumeration of plaques and RT-PCR. PCR neglected inactivation, but also attachment was different, because also inactivated (damaged) virus particles were included.

4.5 Analysis of Breakthrough Curves

In laboratory column experiments and in field studies, usually, microbial tracers, or other surrogates for pathogenic microorganisms are seeded or injected at the starting point for a given duration. The transport of this pulse of microorganisms is monitored by taking samples from an outlet port of the laboratory column or from a monitoring well in the field for the duration of the experiment. The measured concentrations (y-axis) are plotted as a function of time (x-axis) making up a breakthrough curve (BTC). Prior to the seeding of a suspension with one or more microorganisms, it is good practice to inject a conservative chemical tracer, such a sodium chloride, to test the column packing and to determine water flow velocity and dispersion. The arrival time, height and shape of the BTC are determined by the transport processes. Transport parameter values can be estimated by fitting the transport equation to the BTC data. Model equation (1) is implemented in different forms in the HYDRUS-1D software package (Šimůnek et al., 2008) (freely available at http://www.pc-progress.com/en/Default.aspx?hydrus-1d). With HYDRUS-1D, the one-dimensional model described above can be used in several ways. It can be assumed that

the soil has two sorption sites, S_1 and S_2, each having their own attachment and detachment constants. Alternatively, these two sites can be used to describe straining and attachment, respectively, or, it can be assumed that one sorption site represents sorption to the solid phase, while the other the removal of particles by their attachment to the air-water interface.

HYDRUS-1D can be used to fit BTC data as well to simulate BTCs. Figure 5 presents simulated BTCs in order to explain how the transport processes determine arrival time, height and shape the BTCs. BTC1 (top left) represents the BTC of a conservative salt tracer (no attachment, no decay) passing a water-saturated sand column. Hence, the maximum breakthrough concentration $C_{max}$is a high as the seeding concentration C, C_{max}/C=1. The deviation of the climbing limb of BTC1 from a vertical line is due to dispersion. The arrival time corresponds to the time at which the BTC has reached C/C=0.5 Porewater velocity can be derived from the transport length and the arrival time. From the discharge rate of the column and the porewater velocity, the saturated water content of the column can be estimated. BTC2 (top left) represents irreversible attachment of a microorganism (ka=1, no detachment, no decay). The difference with BTC1 is that due to attachment C_{max}/C<1. Note that the same effect on the BTC may be caused by die-off of the microorganisms in the water phase. In fact, this die-off and attachment are indistinguishable in this kind of experiment. It is, therefore, necessary to determine die-off in the water phase in a parallel batch experiment with only water and the microorganism. C_{max} of BTC2 is constant for almost the duration of the seeding, meaning that steady state conditions apply. In BTC3 (top left) attachment is reversible, i.e. detachment occurs. The value of k_d is one-tenth of k_a. For microorganism, often k_d<k_a (Schijven et al., 2002). Because detachment is relatively small, there is no apparent difference between BTC2 and BTC3. However, at the bottom left, these BTCs are plotted with the concentration on a logarithmic scale. Now, the difference between BTC2 and BTC3 is apparent. BTC3 exhibits a so-called tail due to detachment. BTC2 does not have a tail. The height of the tail is mostly determined by k_d. At higher values of k_d, it takes longer to reach a steady state condition. In BTC4 (right), there is also decay of attached microorganisms. On a linear scale, there is no difference with BTC3, but on a logarithmic scale, the tail of BTC4 (bottom right) declines linearly. Eventually, the tail will decline faster, when the column reaches depletion of retained microorganisms. The slope of this declining tail corresponds with the value of the decay coefficient for attached microorganisms. In BTC5, blocking occurs. Due to blocking, attachment slows down with time. This is reflected in an increasing plateau of the BTC. This increase may be subtle and overlooked, and is more pronounced when the seeding duration was longer.

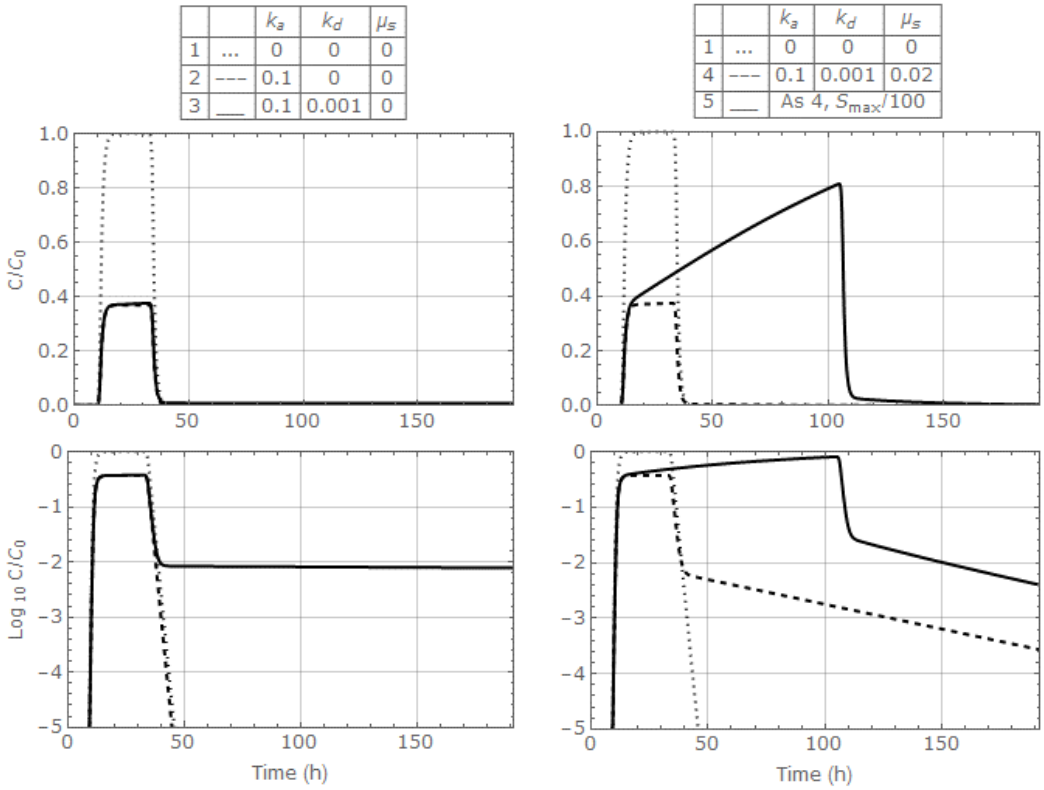

Figure 5. Breakthrough curves of microorganisms

5.0 Microbial Transport in Heterogeneous Subsurface Media

5.1 Transport Determinants

Many subsurface media (e.g. structured soils, alluvial sandy gravel, fractured rocks, karst aquifers) are structurally heterogeneous in their water-conducting abilities. Microbial transport through heterogeneous subsurface media is often under the influence of preferential flow, and in some cases (e.g. open-framework gravels, fractured and karst aquifers), it is the predominating process in microbial migration.

Microbial contaminants can rapidly travel through preferential flow paths (e.g. macropores, cracks, permeable lenses, open-framework zones and fractures). When microbial transport occurs along highly permeable pathways where the groundwater velocity is relatively high, much of the sorptive capacity of the medium is bypassed, resulting in little microbial removal.

5.1.1 Determination of preferential flow paths

Various techniques can be used to determine preferential flow paths in heterogeneous subsurface media. These include slug tests, borehole dilution tests, borehole flow meters, natural and forced gradient salt and dye tracer tests, smoke tests and heat tracer tests. Of all the above techniques, dye tracer approach provides the best visual aid in determining preferential flow paths. Horizontal and vertical flow paths can be very effectively determined by a combined use of dye tracers and passive dye-receptors that are attached to a string/wire and placed in various depths within monitoring wells. A number of dye-receptors have been used for this purpose, such as charcoal packets (Herring, 1999; Toran et al., 2007), resin bags (Pang et al., 1998; Close et al., 2002), activated coconut charcoal and unbleached cotton (Mull et al., 1988). The absorbed dye in the receptors can be then visualized or chemically extracted later on if necessary.

5.1.2 Effect of pore-size exclusion

Like other colloids, when microbes travel through heterogeneous media, they travel primarily through continuous large pores (macropores) or cracks, where flow velocities are the highest. Microbial particles are excluded by small pores (Figure 6). In contrast, solutes would travel through all pores. As a result, microbial transport is often faster than a nonreactive solute tracer when comparing their mean velocities. Due to a reduced pore-network, the effective porosity accessible for microbial transport is reduced and microbial transport is less dispersive than a nonreactive solute tracer. This is reflected in the earlier arrival, sharper and narrower concentration breakthrough curves of microbes than those of nonreactive solute tracers (Figure 7). This is a typical phenomenon frequently observed in the tracer experiments conducted in structured soils and heterogeneous aquifers.

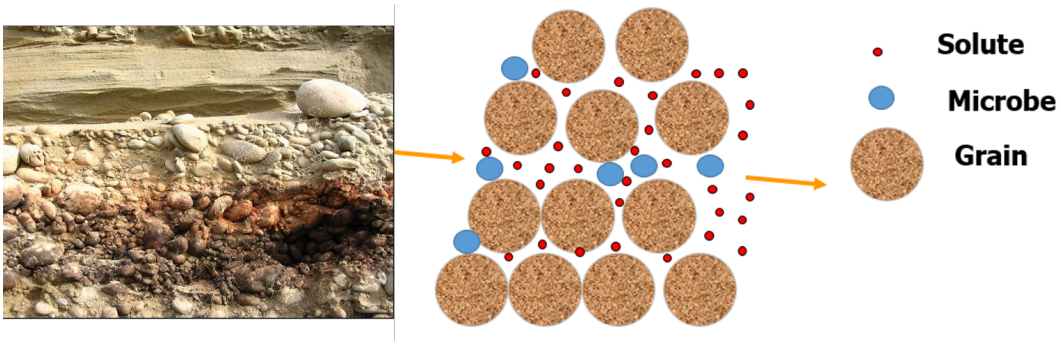

Figure 6. Illustration of pore-size exclusion in microbial transport through heterogeneous porous media

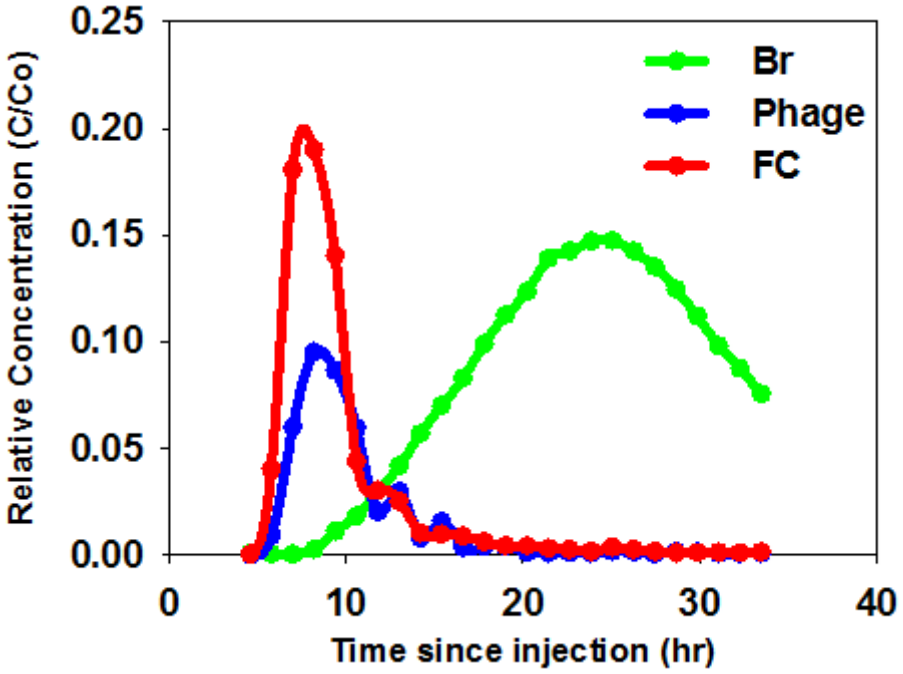

Figure 7. Velocity enhancement of microbial transport in structured soil under effluent irrigation (modified after Pang et al., 2008)

The extent of velocity enhancement is directly related to the degree of heterogeneity in media structure and the size of microbes. For example, microbial transport can display a much greater degree of velocity enhancement in fractured aquifers than in alluvial gravel aquifers due to a higher degree of heterogeneity associated with the fractured aquifers (Table 10). In contrast, pore-size exclusion is not usually observed in homogenous sand aquifers. Generally, the degree of size-exclusion is positively related to microbial size, i.e. bacteria move faster than viruses (Sinton et al., 2000). However, although viruses are smaller in size, they could possibly travel faster than bacteria if they are attached to colloidal particles larger than bacteria. For example, in the field study of Sinton et al. (1997), F-RNA phages were found to travel 1.5 times faster than faecal coliforms, probably due to the attachment of F-RNA phages to the large particles in the sewage effluent used in the experiment.

Table 10. Velocity enhancement of virus transport in heterogeneous aquifers

Aquifer	x (m)	Virus	Virus Velocity (m/d)	Conservative Solute Tracer	Solute Tracer Velocity (m/d)	Virus Velocity Enhancement Factor=V_{virus}/V_{solute}	Reference
Fractured clay till	5	PRD1	2 to 5	Br	0.01 to 0.07	71 to 200	McKay et al. 1993
	5	MS2	2 to 5	Br	0.01 to 0.07	71 to 200	
	4	PRD1	2 to 5	Br	0.01 to 0.07	NR	
	4	MS2	2 to 5	Br	0.01 to 0.07	NR	
Fractured shale saprolite	18	PRD1	11 to 56	Dye	NR	500	McKay et al. 2000
	18	MS2	11 to 42	NR	NR	500	
Gravel and sand	20	H40/1 phage	53 to 100	Uranium	41 to 90	1.11 to 1.29	Flynn, 2003
Karst limestone	1250	H4 & H40 phage	846	NR	770	1.1	Auckenthaler et al., 2002
Sand and gravel	21.5	PRD1	115	Br	129	0.89	Woessner et al., 2001
	21.5	MS2	147	Br	129	1.14	
	21.5	ϕX174 coliphage	147	Br	129	1.14	
	21.5	Poliovirus	172	Br	129	1.33	

Aquifer	x (m)	Virus	Virus Velocity (m/d)	Conservative Solute Tracer	Solute Tracer Velocity (m/d)	Virus Velocity Enhancement Factor=V_{virus}/V_{solute}	Reference
Sand and gravel	14 to 163	Phage T7	11 to 205	NR	NR	3.15	Rossi et al., 1994
	14 to 163	Phage f1	11 to 132	NR	NR	3.15	
Sandy gravel	7.5 to 40.5	MS2	23 to 39	Br	22 to 30	1.05 to 1.30	Deborde et al., 1999
	7.5 to 40.5	PRD1	26 to 39	Br	22 to 30	1.18 to 1.30	
	7.5 to 40.5	ϕX174 coliphage	18 to 39	Br	22 to 30	0.80 to 1.30	
	7.5 to 19.4	Poliovirus	33 to 45	Br	22 to 30	1.5	

Summarized and analyzed by using data from: Pang, 2009; NR: Not Reported

The above different transport characteristics between solutes and microbes have some important implications. For example, setback distances determined from model simulations based on nonreactive solutes in homogeneous aquifers would not be sufficient when preferential flow paths are present.

The spatial removal rate (λ) or the temporal rate (k) are the most appropriate parameters for describing microbial removal efficiencies in heterogeneous aquifers and for estimating setback distances. Both parameters account for the effects of media heterogeneity and site-specific conditions, because they measure microbial concentration reductions over time and distance.

It is not recommended to use the single-collector factor (η) and collision efficiency (α) for describing microbial removal efficiencies of poorly sorted granular media that have a wide range of particle sizes (e.g. gravel aquifers) and consolidated aquifer media (rock and karst aquifers). This is because the calculations of η and α values assume a single particle size and it does not consider particle size distribution. Indeed, Pang et al. (2005) demonstrated that the collision efficiency values determined from a single particle size were unrealistically high (α>1) for microbial removal in heterogeneous gravel aquifers. The η and α parameters are more suitable for describing removal efficiencies of granular media that have uniform size, such as dune sand.

5.1.3 Implications

Because microbes can travel faster than the nonreactive solute tracers due to size-exclusion, their transport velocities determined from heterogeneous media may represent possible maximum groundwaters velocities. In contrast, groundwater velocities determined from nonreactive solute tracers represent the average values as solutes travel through all pores within aquifer media. For risk analysis and design of monitoring programs, a conservative approach would be to consider the maximum groundwater velocities so that “worst case” contamination events, with regard to extent of contaminant transport, can be better predicted and managed.

5.2 Example Studies in Heterogeneous Subsurface Media

5.2.1 Microbial leaching under dairy shed effluent irrigation in New Zealand soils

Aislabie et al. (2001), McLeod et al. (2004; 2003; 2001) and Pang et al. (2008) studied a variety of key New Zealand soils from 12 field sites to evaluate their abilities to attenuate faecal coliforms and bacteriophages. These included allophanic soil, pumice soil, fine sandy loam, recent sandy soil, silt loam, deep silt loam, shallow silt loam over gravels, silty clay loam, clay loam, clayey silt loam and clayey soil. Leaching experiments using intact vegetated soil cores (40–70 cm long, 46–50 cm in diameter) were carried out in triplicate with pulses of dairy shed effluent spiked with *Salmonella* bacteriophages and a bromide tracer. The studies determined that macropores predominantly control microbial transport in highly structured soils, and that even very small volumes of water cause rapid and significant microbial leaching through bypass flow, particularly in clayey soil and clayey silt loam.

Pang et al. (2008) evaluated many of these experimental data using the HYDRUS-1D mobile-immobile 2-region model. It was assumed that microbial transport is limited to the mobile water region and not all the soil water content is

available for microbial transport. Pang found that compared with the bromide solute tracer, microbial transport exhibited velocity enhancement in most soils, less dispersion and a much lower mobile water content, which is, on average, only 19% of the total water content. The exception was homogenous dune sand with a single-grain structure, in which microbial transport was retarded. The modelling results also suggested that the soil structure, or macroporosity, is the most important factor in microbial transport, while the soil lithology has the greatest effect on microbial attenuation.

Pang et al. (2008) confirmed that the general pattern of predicted mobile water content concurred with the measured macroporosity, and that while this was positively related to leaching vulnerability, it was negatively related to dispersivity. Pang (2009) provides estimated microbial removal rates for these soils.

5.2.2 Microbial transport in coarse gravel aquifers in New Zealand.

Microbial transport in highly heterogeneous alluvial gravel aquifers have been extensively studied in the experiments carried out at 2 experimental sites in the Canterbury Plains, New Zealand. Both sites were historically used for sewage effluent irrigation and had arrays of monitoring wells. A range of microbial indicators (bacteria and *bacillus subtilis* spores, faecal coliforms, *E. coli* J6-2, *E. coli* 2690, MS2, F-RNA phages, somatic phages, T4 coliphage, ϕX174 coliphage) were used in the experiments. The estimated velocity of microbial transport range 50-240 m/day. The injected microbial indicators were observed in down-gradient wells as far as 920 m.

The microbial indicators travelled more rapidly than the conservative solute tracers in these studies, and the microbial breakthrough curves were narrower, left-skewed and did not have the pronounced tail as shown in the breakthrough curves of solute tracers. When the solute tracers showed multiple peaks in their concentration breakthrough curves, the microbes usually emerged at the first peak, regardless of the size of the peak. This sometimes resulted in the velocity of microbial transport being up to 3 times faster than that of the solute tracers. Microbial removal rates derived from these experiments were consistently in the order of 10^{-2} $\log_{10}$/m for clean coarse gravel aquifers and 10^{-3} $\log_{10}$/m for contaminated coarse gravel aquifers.

Coarse-grained, heterogeneous gravel aquifers have poor microbial filtration capabilities, and this, together with their high permeability and the presence of preferential flow paths, enables microbial contaminants to travel hundreds of meters.

If natural attenuation was to be relied on, a transport distance of up to 3.9 km in a contaminated coarse gravel aquifer would be necessary to achieve a 7 $\log_{10}$ reduction in the microbial concentration, which is impractical. Therefore, treating effluent to a high specification is vital before it is discharged into especially coarse gravel aquifers.

6.0 Groundwater Protection

In the fourth edition of the World Health Organisation (WHO) Guidelines for Drinking Water Quality (2011), it is stated that securing the microbial safety of drinking-water supplies is based on the use of multiple barriers, from catchment to consumer, to prevent the contamination of drinking water or to reduce contamination to levels not injurious to health. Safety is increased if multiple barriers are in place, including protection of water resources, proper selection and operation of a series of treatment steps and management of distribution systems (piped or otherwise) to maintain and protect treated water quality. The preferred strategy is a management approach that places the primary emphasis on preventing or reducing the entry of pathogens into water sources and reducing reliance on treatment processes for removal of pathogens (WHO, 2011). In the case of groundwater as source for drinking water, multiple treatment barriers may not be in place, or even not desired. In many countries, the abstracted groundwater does undergo additional treatment, usually in the form of chlorine disinfection. The latter treatment is undesirable because of its adverse effect through the formation of disinfection by-products that may be carcinogenic (WHO, 2011), and for that reason it is not practiced in the Netherlands.

To prevent microbial contamination of groundwater, sources of contamination should be kept at such a distance from the pumping well that the abstracted groundwater complies with a health-based target concentration. Such a setback distance may allow for adequate reduction of pathogen concentrations by means of the natural attenuation processes in the subsurface. This leads to the definition of protection zones within which sources of faecal contamination, such as sewers, septic tanks, and manure depots are not allowed. For protection against pathogens, usually a zone based on groundwater travel time is applied, and in several countries this is a travel time of 50–60 days, e.g. in Austria, Denmark, Germany, Ghana, Indonesia, UK (Chave et al., 2006). Also, in the Netherlands protection of groundwater wells is still based on the assumption that a travel time of 60 days is sufficient for die-off of pathogenic bacteria in contaminated groundwater to the extent that no health risks would exist (Schijven et al., 2006). However, it is known that pathogenic viruses and protozoa as well as bacteria can survive much longer than 60 days in soil and groundwater (Pedley et al., 2006). Given the persistence of pathogens, a protection zone of 60 days may not be sufficient to protect public health.

Often, for various reasons, removal of microorganisms during subsurface transport is higher during the first meters and much less thereafter, which is not seen in column experiments. The reasons for this nonlinear removal, may be (a combination of) population heterogeneity of the microorganisms, the presence of redox zones (Schijven et al., 2000), variable water saturation, soil heterogeneities (Pang, 2009). Population heterogeneity implies that all microorganism particles are not identical (Foppen and Schijven, 2006; Schijven and Hassanizadeh, 2000). Redox zones exist in situations where oxygen saturated water infiltrates groundwater. The so-called oxic

zone is near the point of infiltration. The oxygen in the water oxidises soil minerals. Farther from the infiltration point oxygen levels have therefore decreased. Schijven et al. (2000) showed that virus sticking efficiencies ranged in the order of magnitude from 10^{-3} to 10^{-5}, spanning redox zones from oxic to anoxic. The low sticking efficiency value of 10^{-5} for anoxic conditions was confirmed by Van der Wielen et al. (2008). This range of sticking efficiencies corresponds with effective to ineffective virus removal by attachment.

Following WHO drinking water guidelines (WHO, 2011), the current Dutch legislation for drinking water requires that the risk of infection by a pathogen due to drinking water consumption may not exceed the level of one per 10,000 persons per year (Schijven et al., 2006), corresponding to less than one pathogen in one million litre of water. In order to evaluate whether shallow unconfined sandy aquifers, that can be considered vulnerable for those reasons, are adequately protected by a 60-days zone, i.e. whether the abstracted groundwater in the presence of a virus source still complies with the infection risk level, Schijven et al. (2006) calculated protection zones for shallow unconfined sandy aquifers, considering only horizontal transport from the contamination source to the production well. Viruses leaking from a sewage pipe were simulated with slow virus inactivation and a low rate of virus attachment to the sand. They concluded that protection zones with a travel time of 1 – 2 years instead of 60 days would be needed in many cases. Here Schijven et al. (2006; 2010) followed the precautionary principle with the low value for virus attachment in terms of a sticking efficiency of 10^{-5} and a virus inactivation rate coefficient of 0.02 day^{-1}. Setback distances may be much smaller if there is more attachment and inactivation.

The model for calculating setback distances of groundwater protection zones (Schijven et al., 2010) has been implemented in an interactive cool, named QMRA*well*. The tool calculates a setback distance that is required to not exceed a certain virus concentration level at a single groundwater production well, or it calculates the virus concentration at the well for a given setback distance and virus concentration in the source. This model is applicable when radial flow of water towards a continuously pumping well dominates water flow due to a natural gradient (usually at a pumping rate of at least 1000 m^3/day). Among pathogens, viruses are critical for groundwater contamination, because they are excreted in large numbers with the stool of infected individuals, are stable in the environment, travel great distances in groundwater and are highly infectious. Even at very low undetectable concentrations in drinking water, ingestion commonly leads to diarrhoea and vomiting, or to more severe illness or even death (Borchardt et al., 2012; Schijven and Hassanizadeh, 2000). Virus concentrations are reduced by dilution with water flowing from all directions to the well, by attachment, with sticking efficiency *a* as input parameter and by first-order inactivation. The aquifer domain may consist of several permeable sandy layers that may be separated by aquitards (clay layers). The required concentration reductions and the virus concentration at the well are determined by a health based target, in this case infection risk per person per year. For protection against virus contamination as sticking efficiency $a=10^{-5}$ and inactivation rate coefficient $\mu=0.023$ day^{-1} are default conservative values, representing virus inactivation at about 10°C and unfavourable, anoxic conditions for attachment, respectively (Schijven et al., 2006). In QMRA*well*, virus detachment is neglected. In field studies on virus transport, it is commonly observed that under constant conditions, virus detachment is much slower than attachment. At temperatures of 10°C or lower, removal of a persistent virus is mostly determined by attachment. At higher temperatures, inactivation becomes dominant. Note that attachment and inactivation are the most important parameters determining virus transport in the subsurface and that transport predictions are highly sensitive to the values for attachment and inactivation (Schijven et al., 2006). In addition to temperature, virus inactivation also depends on hydrochemical conditions. Schijven et al. (2016) developed an empirical formula to predict inactivation of bacteriophage PR1 as a conservative model virus for the following conditions: 9.5°C and 12°C, pH4 – pH8, sodium concentrations of 1, 10 and 20 mM, and calcium concentrations of 0.5, 1.5, and 3 mM. Model predictions are within ± 0.4 $\log_{10}$ (0.4 – 2.5 times) virus concentration reduction. Inactivation rate of PRD1 was found to increase with increasing temperature and increasing sodium and calcium concentrations, and to be lowest between pH 6.5 – pH 7.5. The lowest value of the inactivation rate of PRD1 predicted by this model at pH7 and 1 mM sodium is about 0.023 day^{-1}.

As observed in column experiments under dynamic hydraulic and chemical conditions (transients), attached viruses may detach quickly and lead to peak breakthrough concentrations that are even higher than the initial concentration. Such transient conditions may be caused by rain fall events affecting ionic strength, water flow, and water saturation simultaneously (Torkzaban et al., 2006a, Torkzaban et al., 2006b; Zhang et al., 2013).

Most colloid transport studies have considered clean bed conditions by assuming a constant attachment rate and infinite retention capacity. In reality, soil and aquifer material will always have a finite retention capacity and only a small fraction of the solid surface may contribute to colloid retention even under favourable attachment conditions (Sasidharan et al., 2014). Blocking of available retentions sites, like by other much more abundant particulate organic matter, decreases the attachment rate over time and enhances the transport of colloids. For predicting virus transport at field scale, blocking may need to be taken into account.

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