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TRANSPORT AND FATE OF *ESCHERICHIA COLI* IN UNSATURATED POROUS MEDIA

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Nomenclature and Abbreviations

Units

L	Length
M	Mass
N	Number of particles
T	Time

Variables and coefficients

c	Concentration of cells [NL^{-3}]
C_{frac}	Fraction of transported cells
c_0	Influent concentration [NL^{-3}]
C_{ina}	Number of inactivated cells per volume fluid [NL^{-3}]
C_{Wim}	Number of immobile cells per volume fluid [NL^{-3}]
C_{Wm}	Number of mobile cells per volume fluid [NL^{-3}]
D_{diff}	Molecular diffusion [L^2T^{-1}]
D_z	Longitudinal dispersion coefficient [L^2T^{-1}]
h	Pressure head [L]
$iend$	End time of pulse [T]
$istart$	Start time of pulse [T]
K_d	Empirical Freundlich distribution coefficient [L^3M^{-1}]
k_{dep}	Deposition rate to the immobile phase [T^{-1}]
k_{ina}	Temporal first-order inactivation coefficient [T^{-1}]
$K(h)$	Hydraulic conductivity (unsaturated) [LT^{-1}]
k_{rel}	Release rate from the immobile phase [T^{-1}]
k_{rem}	Total temporal first-order removal coefficient [T^{-1}]
k_{rems}	Total spatial first-order removal coefficient [L^{-1}]
k_{ret}	Temporal first-order retention coefficient [T^{-1}]
$K_r\ h$	Relative hydraulic conductivity
K_s	Saturated hydraulic conductivity [LT^{-1}]
m	van Genuchten parameter
m_f	Freundlich coefficient
n	van Genuchten parameter
P_{rel}	Peak relative effluent concentration
q	Darcy velocity [LT^{-1}]
$Q\ t$	Flow rate [L^3T^{-1}]
R	Retardation factor
t	Time [T]
v	Average interstitial pore velocity [LT^{-1}]
z	Height above a datum (positive upwards) [L]

Greek letters

α	van Genuchten parameter [L^{-1}]
α_L	Longitudinal dispersivity [L]
Θ	Saturation
θ_r	Residual volumetric moisture content
θ_s	Saturated volumetric moisture content
θ_W	Volumetric moisture content
Λ_{fate}	Bacterial fate reaction rate, source-sink term
ρ_b	Filter media bulk density [ML^{-3}]

Abbreviations

BTC	Breakthrough curve
CFT	Colloid Filtration Theory
CFU	Colony-Forming Unit
DVLO	Derjaguin and Landau, Verwey and Overbeek
E. coli:	*Escherichia coli*

Figure colors

	Bacteria
	Colloid
	Gas
	Liquid
	Solid

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List of Papers

I. Engström, E., Thunvik, R., Kulabako, R., Balfors, B. (2011): *Escherichia coli* transport and fate in unsaturated porous media: a literature review of experimental findings and theories relating to processes, models and influencing factors, under review in Critical Reviews in Environmental Science and Technology.

II. Engström, E., Balfors, B., Thunvik, R. (2010): Modeling Bacterial Transport and Removal in a Constructed Wetland System. Proceedings of the COMSOL Conference, November 17-19, 2010, Paris.

III. Engström, E., Thunvik, R., Balfors, B. (2011): Predicting the transport and fate of *Escherichia coli* in unsaturated sand filters, Submitted to Journal of Contaminant Hydrology, November 2011.

ABSTRACT

The unsaturated zone could provide an effective barrier against pathogenic microbes entering the groundwater. Knowledge relating to microbial fate in this zone is therefore important for increased understanding of groundwater vulnerability. This thesis examines the published literature that is related to the transport, retention and survival processes that apply to the fecal indicator bacterium *Escherichia coli* in unsaturated porous media. The main focus concerns the research findings under steady-state flow in homogeneous filter media, and under unfavorable attachment conditions, which are the most common in the natural environment. Experimental results in the literature for the pore-, column- and field-scale are examined and compared to commonly applied theories and modeling approaches. An analysis of the main factors that influence attenuation and biofilm formation is provided. Further, the findings are illustrated in a model of an unplanted, vertical flow constructed wetland. The results indicate that retention at the solid-air-water interface is a major attenuation process. In addition, they suggest that the flow velocity (as dependent on the grain size and the saturation) is a key influencing factor. However, it has not yet been established how the research findings relating to the main processes and influencing factors can be incorporated into predictive models; in the literature, a multitude of models have been proposed and alternative theories could describe the same observation. In this study, the transport and fate of *Escherichia coli* in different sand filters is, therefore, modeled using various literature models - derived under similar experimental conditions - in order to assess the possibility to compare and generalize the equations, evaluate their implications considering the different saturation settings and filter depths, and to define the spectra of the reduction efficiencies. It is discovered that the bacterial attenuation behaviors vary largely. This calls for clarification regarding the underlying processes. Future research is also recommended to include the effects of structured filter media and sudden changes in the flow rate.

Key words: Contaminant transport modeling; Bacterial fate; Unsaturated zone; Retention; Straining; *Escherichia coli*

INTRODUCTION

Groundwater is a very important source of drinking water. However, pathogenic microbes can enter into the groundwater from the disposal of excreta using pit-latrines, the discharge of wastewater and -material to land, and the leakage of sewer lines (Crane and Moore 1986; Scandura and Sobsey 1997; Azadpour-Keeley and Ward 2005). Studies have shown that half of the tested drinking water wells in the U.S. contain fecal pollution, which is estimated to cause up to 5.9 million illnesses per year (Macler and Merkle 2000). Moreover, diarrheal disease was estimated to be responsible for 1.6 million deaths in 2003 (WHO 2003). To a large extent (88%) this was attributed to unsafe water supplies, sanitation and hygiene, and the most affected group was reported to be children in developing countries. The poor population in these countries often relies on shallow groundwater sources and on-site sanitation (Nsuguba 2004). For an improved understanding of groundwater vulnerability and the development of health regulations, knowledge relating to microbial transport and fate is essential (see, *e.g.*, Jin and Flury (2002) and Emelko and Tufenkji (2010)). Additionally, an improved insight into the processes associated with bacterial attenuation can play a role in the bioremediation and biofacilitated transport of pollutants (Schäfer *et al.* 1998). As water generally becomes scarcer and the demand for fresh water intensifies, degraded water - such as storm water, agricultural wastewater and gray water- could provide a valuable resource and its use is likely to increase in the future (Hamilton *et al.* 2007; O'Connor *et al.* 2008). Sand filters and constructed wetlands have the potential to provide effective and inexpensive wastewater treatment (Falvey 1997; Brix and Arias 2005). However, there must be a greater understanding in relation to their removal capacity in order to provide improved guidelines for their construction and use.

Project inspiration and scope

The motivation behind this project was the results in a case study by Kulabako *et al.* (2007), relating to a spring in an inhabited wetland in a peri-urban (slum) area in Bwaise III Parish, Kampala, Uganda: the spring water was reported to be contaminated with thermotolerant (fecal) coliforms. Further, the use of water from similar, groundwater-fed, so called, protected springs in this region has been linked to the incidence of acute diarrhea and cholera (Nasinyama *et al.* 2000; Howard *et al.* 2003). The withdrawal of water from these springs is nevertheless common in this part of Kampala, as the income level is low and the water is free of charge. Onsite visual observations in March 2010 revealed that the risk for anthropogenic pollution of the shallow aquifers upstream of the spring is high (Fig. 1): the spring is situated near a road, from which cattle droppings and petrol can percolate into the ground and dissipate into the groundwater that feeds it; there is no upstream storm water diversion ditch; the surrounding soil is made up of roughly half a meter of litter; residences are located approximately 15 meters upstream; and no authority is looking after any encroachment to the top of the protection area by either humans or cattle. During the field visit, questions were raised: how could one predict the bacterial concentrations in the spring and the timescales for the cells to reach the groundwater in the dry and the wet seasons? Which physical, chemical and biological processes govern bacterial transport to the groundwater? How could the attenuation capacity of the protection area be improved, *e.g.*, with other filter materials or constructions?

These questions provided the inspiration for research that would, if possible, include: predictions of the bacterial processes in the unsaturated subsurface area upstream of the spring; an evaluation of various protection area designs; and, contributions to decision support regarding the delineation of a groundwater protection area and other spring protection measures. However, these ambitions were linked to methodological challenges: the sources of the anthropogenic pollution are innumerable in Bwaise III considering its tumultuous characteristics, and controlled experiments are practically impossible in the area. Moreover, legal restrictions apply to the practice of injecting bacteria in a populated region. As an alternative approach, a model of the whole catchment could be constructed to make recommendations relating to, for example, a protection area delimitation; still, measurements to calibrate and validate such a model in Bwaise III could not be practically obtained within the scope of this thesis. A more pragmatic approach would, thus, include the design of purely physical models to describe and predict the impact of realistic and relevant, synthetically generated scenarios on a smaller scale, using parameters derived from the literature. In order to construct such models, it is imperative to understand the physics relating to the processes involved and the models that could be used to predict them.

Aim of the licentiate thesis

The insights in the previous section contributed to the specification of the overall aim for this licentiate thesis: to improve knowledge relating to bacterial transport and fate in the upper part of the subsurface. More specifically, the objectives are:

Fig. 1. The protected spring in Bwaise III. Possible, apparent, sources of contamination of the spring are: animals and people walking freely on top of the protection area; black water from the pit latrine of the house to the left; waste water from the car wash to the right; cattle droppings and petrol from the road just behind the three women approaching the spring. Photo: Emma Engström, March 2010.

- to provide an in-depth review of theories and empirical studies in the literature relating to the processes involved in *Escherichia coli* (*E. coli*) transport, retention and survival in the unsaturated zone;
- to evaluate and compare the most important physical, chemical and biological factors that have been reported to influence these processes; and
- to critically analyze the implications and possibilities for the applications of the current, associated modeling approaches.

Previous research

Early literature studies, such as McDowell-Boyer *et al.* (1986) and Yates *et al.* (1988), examined colloidal transport processes in the subsurface in general. Since then, the importance of the gas-water interface, particularly, has been highlighted in experimental studies (Wan and Wilson 1994a; Wan and Wilson 1994b; Wan *et al.* 1994). More recent reviews have qualitatively discussed the physical, chemical and biological factors that influence microbial attenuation (*e.g.*, Jamieson *et al.* 2002; Stevik *et al.* 2004); however, they have not evaluated predictive models. In the saturated zone, findings on microbial deposition and fate have been assessed by Ginn *et al.* (2002), Murphy and Ginn (2000) and Tufenkji (2007), as well as Foppen and Schijven (2006), the latter focusing particularly on *E. coli*. In relation to transport in porous media in general, Shen and Devin (2007) and Sen (2011) have addressed colloids and biocolloids, respectively. The extensive review by Schijven and Hassanizadeh (2000) focused on virus removal under saturated conditions. Keller and Sirivithayapakorn (2007), Keller and Auset (2004) and Bradford and Torkzaban (2008) have particularly addressed pore-scale mechanisms. DeNovio *et al.* (2004) analyzed colloidal transport and retention in unsaturated conditions, in which the focus was on fluctuations in pore water flow rates. In addition, Rockhold *et al.* (2004) reviewed the coupling of microbial transport and other constituent processes, *e.g.* the interaction between microbial growth and the inter-phase exchange of oxygen. With regards to modeling, numerical models have previously been developed to incorporate experimental findings for illustrative purposes (see, for example, Kim *et al.* (2008), Yavuz Corapcioglu and Haridas (1985) and Foppen and Schijven (2006) (bacteria) as well as Bhattacharjee *et al.* (2002) and Schijven and Simunek (2002) (virus), as well as Lewis *et al.* (2004) and Schijven and Simunek (2002). However, none of these have given particular consideration to *E. coli* transport in the unsaturated zone.

BACKGROUND

This chapter summarizes the basic data relating to *E. coli*, unsaturated filter media, and the most important theoretical models applying to flow and transport.

The choice of *E. coli*

This subsection summarizes the rationale for focusing on the bacterium *E. coli*, specially. The most widespread health risks associated with drinking water are diseases whose origin are in the pathogenic microbes that are spread through human and animal excreta (WHO 2008), which always contain fecal coliforms (Pang *et al.* 2008). *E. coli* (Fig. 2) is a recommended indicator of fecal contamination (WHO 2008), as it is well-characterized and easily detected. It is a typical indigenous bacterium in soil and is also a representative of those microorganisms used for soil bioremediation (Chen *et al.* 2010). In addition, *E. coli* has a comparatively high rate of transport (low rate of attenuation), as it is relatively hydrophilic and negatively charged; hence it attaches unfavorably to soil grains under most environmental conditions (Foppen and Schijven 2006). Moreover, it is motile and has a low die-off rate. This supports the use of *E. coli* in models intended for, *e.g.*, decision support regarding protection area delimitation. Under unsaturated conditions, a relatively high amount of experiments have further addressed *E. coli* attenuation, as compared to other microbes (*e.g.*, Jiang *et al.* (2007), Torkzaban *et al.* (2008b) and Chen *et al.* (2010)). For a more detailed discussion with regards to the relevance of focusing on *E. coli*, see Foppen and Schijven (2006). Nevertheless, studies on *E. coli* in the unsaturated zone are as yet rare (in absolute terms); therefore, literature findings relating to the saturated zone, as well as on colloids with characteristics similar to *E. coli* – with regard to size, hydrophily, and surface charge – are also analyzed in this thesis. This latter approach is common; however, the difference in the removal behavior between abiotic colloids and bacteria has not yet been fully clarified; the difference in rheology is, for example, believed to affect attachment behavior (Ohshima and Kondo 1991; Ohshima 1995).

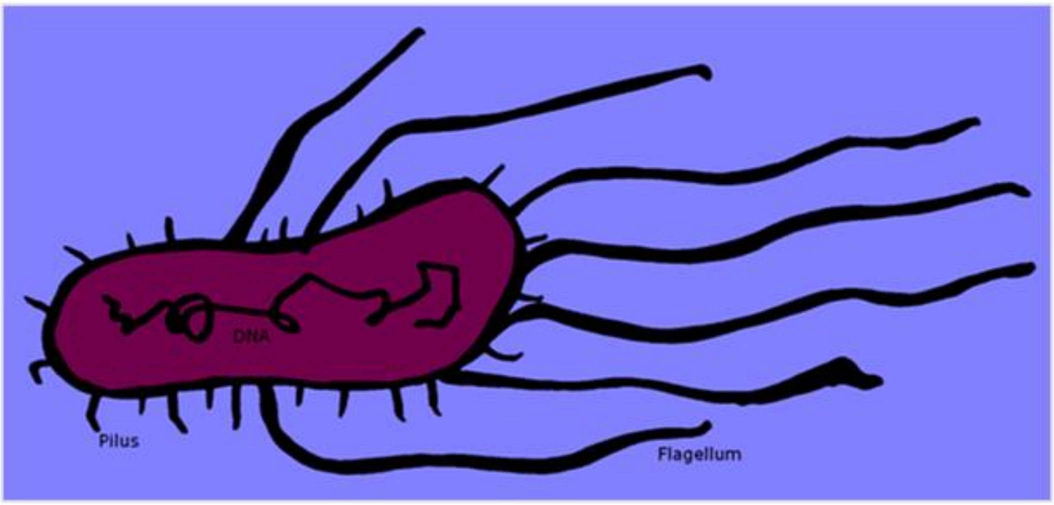

Fig. 2. E. coli with discernible flagella (about 30 nm width), pili (about 20 nm width) and DNA: the cell has an oblong shape. As the flagella rotate counterclockwise the bacteria is pushed forward; however, if at least one flagellum rotates clockwise, the cell is moved in a chaotic, tumbling (Brownian) motion (McClaine and Ford 2002b; Parkinson 2009). The figure is redrawn from Li (2007).

Unsaturated filter systems

Pathogens generally enter the groundwater through the unsaturated zone (Steenhuis *et al.* 2006). Contaminant sources are often located close to the ground surface (Wan and Tokunaga 1997; Schäfer *et al.* 1998). This region could provide an effective barrier against microbes and act as a protection for down-gradient sources (Scandura and Sobsey 1997; Azadpour-Keeley and Ward 2005). Research relating to microbial transport under unsaturated conditions is however rare, particularly at the field scale (DeNovio *et al.* 2004; Burkhardt *et al.* 2008; Pang 2009). In addition to the physical, chemical and biological processes that apply to the saturated zone, removal is complicated by the presence of air (McCarthy and McKay 2004). According to Pang (2009), most studies that have derived setback distances for aquifers only consider microbial transport through aquifers. Pang (2009) concluded that these results are only applicable for the worst-case models when the water tables are close to the bottom of a disposal system, and added that the inclusion of unsaturated zones (in addition to the aquifers) in predictions could significantly reduce the required setback distances.

The most common sand filters are those which are buried (Falvey 1997). The filters are about half a meter deep, and the filter media typically has grains of sizes of 0.3 - 1 mm, which are relatively uniform in size in order to reduce clogging. The hydraulic loading is 0.05 - 0.06 m^3/m^2/day, which, for continuous flow implies that the Darcy velocity is about 0.004 cm/min (Falvey 1997). Sand filters have many advantages: they are less costly to construct than centralized treatment systems; they are energy efficient; the maintenance requirements are low; and the treatment is of high quality (Falvey 1997). Knowledge of the removal potential of sand filters is, moreover, relevant for the understanding of attenuation in vertical flow unplanted constructed wetlands, as studied by, *e.g.*, Vacca *et al.* (2005) and Sleytr *et al.* (2007). These are increasingly being used to handle anthropogenic waster, *e.g.* pathogen removal from wastewater, storm water and sewage (Langergraber and Simunek 2005). It is possible that they could enable there to be efficient sewage water treatment for subsequent discharge into groundwater (Brix and Arias 2005), and to provide promising low cost filters; however, their removal capacity requires a better understanding in order to provide for improved guidelines with regards to construction and use. For example, little research has hitherto evaluated the removal effectiveness of constructed wetlands with regard to specific pathogens (García *et al.* 2010).

Predictive modeling of flow and bacterial transport in unsaturated porous media

This subsection outlines the models and computer tools that were applied to predict bacterial transport in the current study.

Flow: Richard's equation

In the subsurface, microbes are generally considered to be transported with the water (Schäfer *et al.* 1998). In the vadose zone, flow is typically vertical (Pang 2009). It is comparatively slow (and laminar) and takes place in small, water-filled pores, since it is limited by capillary forces (Bradford and Torkzaban 2008). Bacteria and water could also move in thin films of water that surround the mineral grains and in liquid-filled corners of angular pores (Saiers and Lenhart 2003). Uniform flow is generally modeled using Richard's equation (Richards 1931; Schijven and Simunek 2002; Schwartz and Zhang 2003). In the one-dimensional case, this equation becomes:

$$\frac{\partial \theta_W(h)}{\partial t} = \frac{\partial}{\partial z}\ K\ h\ (\frac{\partial h}{\partial z} + 1) \tag{1}$$

where t [T] is time; $\theta_W(h)$ is the volumetric water content and h [L] is hydraulic head, defined by $h = \psi + \mathrm{z}$, where ψ [L] is the pressure head, negative in the unsaturated zone and positive in the saturated zone and z [L] is the height above a reference level (positive upwards); $K\ h = K_r\ h\ K_s$, where $K_r\ \psi$ is the relative hydraulic conductivity and K_s [LT^{-1}] is the saturated hydraulic conductivity tensor. In the case of steady-state flow, the left-hand side of Richard's equation equals zero. The volumetric water content and hydraulic conductivity are functions of the pressure head, as, e g., described by the Brooks and Corey (1964) and van Genuchten (1980) equations, of which the latter are:

$$\theta_W\ h\ = \theta_r + \frac{\theta_s - \theta_r}{1 + \ \alpha h\ ^{n\ m}},\quad h < 0 \tag{2}$$

$$\theta_W\ h\ = \theta_s,\quad h \geq 0 \tag{3}$$

$$K_r\ h\ = \frac{[1 - \ \alpha h\ ^{n-1}(1 + \ \alpha h\ ^{n})^{-m}]^2}{(1 + \ \alpha h\ ^{n})^{m/2}} \tag{4}$$

where $m = 1 - \frac{1}{n}\ (n > 1)$. A high α implies that the soil desaturates at low suctions, and a high n that the water retention curve has a steep slope, which typically applies to coarse filter media. Lastly, saturation, Θ, equals $\frac{\theta_W - \theta_r}{\theta_s - \theta_r}$. However, it is not possible to deterministically and completely describe a geologic medium, since it is not an engineered system (Tsang *et al.* 1994). To include finer structures, one can apply stochastic modeling techniques, which, moreover, provide estimates of the uncertainty of the model parameters (Tsang *et al.* 1994). Molin and Cvetkovic (2010) included spatial variability of parameters in predicative models of microbial transport in the saturated zone. Studying spatial variability of various unsaturated flow parameters, Russo and Bouton (1992) reported that K_s and α, as compared to the other parameters in the van Genuchten equations above, showed a high degree of variability in space. Nevertheless, for predictions relating to a specific system, the inclusion of stochastic spatial variability demands that the probability distributions of the relevant parameters are well defined.

Bacterial transport: the extended Advection-Dispersion Equation

The most commonly used equation for describing mass transport in aqueous systems at a representative element volume scale is the Fickian-based advection-dispersion equation, where flux is proportional to the concentration gradient (Reddy *et al.* 1981; Schwartz and Zhang 2003). It could be extended to account for a bacterial reaction-rate source-sink term, Λ_{fate} (Rockhold *et al.* 2004), which, per bulk volume porous media, develops into:

$$\theta_W \frac{\partial C_W}{\partial t} = \theta_W \nabla \ D \nabla C_W \ - \theta_W \nabla \ v C_W \ + \Lambda_{fate} \quad (5)$$

where D [L^2T^{-1}] is the hydrodynamic dispersion coefficient; C_W [NL^{-3}] is number of particles per liquid volume; and v [LT^{-1}] is the average interstitial (pore) water velocity. The velocity term in eq. 5 can be calculated from Richard's equation, and in this way transport is coupled to flow. For two dimensions, in a locally isotropic medium, the hydrodynamic dispersion coefficient is commonly defined as:

$$D_{L,T} = \ v \ \alpha_{L,T} + D_{diff} \quad (6)$$

where $D_{L,T}$ [L^2T^{-1}] is the longitudinal or transverse dispersion; the first term on the right-hand side describe the mechanical dispersion and v is the pore-water velocity and $\alpha_{L,T}$[L] is the longitudinal or transverse dispersivity, which depends on the movement of water around soil grains and hence, the soil heterogeneity; and D_{diff} [L^2T^{-1}] is the molecular diffusion, *i.e.* the spreading from high to low concentration areas, generally described using the Stokes–Einstein equation (Yao *et al.* 1971; Baumann *et al.* 2010). Mechanical dispersivity is dependent on scale, and, as a rule of thumb, is according to $\alpha_L = 0.1L$, where L is the experimental length scale, *e.g.*, the distance between an injection point and a well (Baumann *et al.* 2010). However, under unsaturated conditions, considerable dispersion of colloid fronts have been registered, which is attributed to the fact that colloids that are not deposited are thus are forced to travel through more complex paths in the vadose zone than in the saturated one (Keller and Sirivithayapakorn 2004). In summation, dispersion in the unsaturated zone is, as yet, not fully clarified, due to a lack of consistent and comprehensive data sets (Toride *et al.* 2003). When considering the transport of bacteria, the concentration is generally measured in relation to the amount of particles, rather than the mass (Crane and Moore 1986; Sun *et al.* 2001; Tufenkji 2007). With regards to bacterial fate in porous media, the main mechanisms involved are: bacterial decay and growth, as well as deposition and release, which relate to the exchange of colloids between the gas, liquid and solid phases (Vega *et al.* 2003). However, the models applied to describe bacterial behavioral processes, Λ_{fate} , have, as yet, been debated.

Defining model parameters

Model parameters could be assigned based on a knowledge of the underlying physical processes that govern the system (physical models), or through calibration (calibrated models). In the former, the parameters are defined so that they have a physical meaning and the equations are typically founded on continuity of mass or momentum. Experimental measurements can be made to define individual parameters. However, these have to account for the heterogeneities in the modeled system, and, typically, spatial averaging or approximate representations which incorporate time dependencies are applied (Tsang *et al.* 1994; Åkesson 2010). In calibrated models, the parameters are defined by means of a statistical best fit of the observed data to a few governing equations, which often represent several physical processes. Parameters are often system specific; hence, the transfer of parameters between different systems is limited. In column studies of bacterial transport, the advection-dispersion equation, eq. 5, is generally assumed to apply. Typically, dispersion is obtained by means of a best fit of the advection- dispersion equation to tracer breakthrough observations, and the reaction parameters are determined by fitting some assumed reactive equation, Λ_{fate}, to the bacterial breakthrough curve. This statistical approach places limitations upon the applicability of models to other experimental systems; parameters derived from previous studies are rarely, if ever, applied to predict results in a new experimental setting. This complicates there being any meaningful comparisons, and thus the knowledge with regards to the possibilities of generalizing the equations and parameters is limited - a theme that is discussed more thoroughly in *Paper III*.

Computer tools used to solve flow and transport equations

Due to its nonlinear properties, Richards' equation is generally solved numerically, and, in the literature, a wide range of numerical tools have been applied to solve the equations for flow, transport, as well as for various types of retention and survival processes, *i.e.*, different expressions for Λ_{fate}. They vary with regard to: the processes considered; the discretization technique applied (finite difference or finite element methods); the ability to couple different physical processes; the boundary conditions allowed; the dimensionality and resolution; and to the degree of user flexibility. Several reviews have evaluated the software that could be used to describe colloidal transport and fate. For example, Azadpour-Keeley and Ward (2005) discussed various computer tools that have been designed for virus deposition and inactivation in the subsurface, some of which can also be used for bacterial modeling, particularly HYDRUS-1D (Simunek *et al.* 2005) and HYDRUS-2D (Simunek *et al.* 1999a). Simunek *et al.* (2003) compared models especially designed for non-equilibrium and preferential flow and transport in the unsaturated zone. Rockhold *et al.* (2004) discussed computer tools relevant for microbial flow and transport processes in porous media and stressed that the majority of them were only applicable to one-dimensional modeling in saturated filter media; they determined that only a few models could account for the porosity and permeability which results from the accumulation of biomass that occurs in filter media. Previous microbial modeling transport studies under saturated and unsaturated conditions have included: HYDRUS-1D (Simunek *et al.* 2005), applied by, *e.g.*, Gargiulo *et al.* (2007) and Torkzaban *et al.* (2008a); HYDRUS-2D (1999a), applied by *e.g.* Schijven and Simunek (2002); MODFLOW (Winston 2010) and MT3DMS (Zheng and Wang 1999), applied by, *e.g.*, Foppen and Schijven (2006); PHREEQC (Parkhurst and Appelo 1999), applied by, *e.g.*, Foppen *et al.* (2008); STANMOD (Simunek *et al.* 1999b), applied by Jiang *et al.* (2007); as well as AQUASIM (Reichert 1994), applied by, *e.g.*, Schäfer *et al.* (1998). Another related computer tool is TOUGH, which has been designed for geothermal applications in particular (see Pruess (2004)).

METHODOLOGY

In this thesis the methodology primarily includes a literature review, which is mainly based on published scientific articles. Numerical modeling has additionally been applied in order to quantitatively analyze and illustrate the review results.

A literature review - the foundation for further research (*Paper I*)

Recent years have seen important progress in the understanding of microbial transport and fate in porous media (see *e.g.* Emelko and Tufenkji (2010)). Related computer tools have undergone the same development; however, at the same time, ground water microbial contamination is clearly still an urgent problem in many parts of the world, particularly in urban areas within developing countries (Trefry and Haque 2010).The recent findings in the two disciplines could be combined in the construction of predictive models. Adequately constructed models could provide important tools when quantitatively assessing microbial transport and removal (Schijven and Hassanizadeh 2000; WHO 2008), and could provide valuable information to practitioners within the fields of resource management, land-use planning and risk analysis, *e.g.*, government agencies, regional authorities, and consultants (Pang *et al.* 2008). The improvement of predictive models is particularly relevant for water usage in densely populated areas in the developing world, where access to local data is often limited, controlled experiments are difficult, and there is an acute requirement for scientific evidence in order to improve current water protection policies; models that are based on laboratory data have the potential to provide a better understanding of the outcomes with regards to various scenarios relatively cheaply, *e.g.* the removal efficiency of a particular protection area design (ARGOSS 2001; Ausland *et al.* 2002; Howard *et al.* 2003; WHO 2009; Emelko and Tufenkji 2010; Trefry and Haque 2010). A thorough understanding of the relevant model designs and parameter values are crucial in relation to the success of such modeling (Azadpour-Keeley and Ward 2005). Tsang *et al.* (1994) stated that, in order to make predictive assessment of transport in heterogeneous media, the applicable physical, biological and chemical processes – relating to flow, transport and reactions – must be well understood and reflected in the governing equations. Finally, Brix and Arias (2005) argued that an improved knowledge in relation to microbial transport processes could contribute to guidelines regarding treatment requirements concerning single dwellings in rural areas. Therefore, the methodology in the first part of this thesis includes a critical, comparative analysis of the published findings relating to the transport, attachment, straining and survival processes that apply to *E. coli* in unsaturated porous media. Experimental results for the pore-, column- and field-scale are examined and compared to the commonly applied theories and modeling approaches. An analysis of the key influencing physical, chemical and biological factors that influence attenuation, as well as the impact of biofilm on *E. coli* attenuation is provided. Additionally, the main focus is on the processes under common environmental conditions, which are, generally, unfavorable for attachment: negatively charged filter media (Foppen and Schijven 2006), as well as a transporting solution of low ionic strength (Yang *et al.* 2007; Wang and Zhou 2010) and approximately neutral pH (Schijven and Hassanizadeh 2000). Most cited studies regard steady-state flow in homogeneous media, since theories and processes under these conditions - although simplified - are, as yet, still under debate.

Numerical modeling for quantitative analyses (*Paper II* and *Paper III*)

The results in the review determined the two subsequent studies. Simulations were performed to illustrate the review results in a simple, unplanted, vertical flow constructed wetland (*Paper II*), and to compare and generalize literature models, as well as to evaluate the possibility to predict the removal efficiency of *E. coli* in a simple sand filter system (*Paper III*). In both of these studies, the flow was assumed to be homogeneous and steady-state, thus, it was modeled using Richard's equation: eq. 1, with the left-hand side equal to zero. This was coupled to the advection-dispersion equation and various different bacterial fate equations (eq. 5). It was beyond the scope of *Paper II* and *Paper III* to quantify the removal processes that occur due to the activity of other microbes, such as biofilm formation, even though they are likely to be significant in sand filter systems; the predictions would be complicated by the fact that these processes are highly dependent on the assumptions made on the surrounding environment. The governing equations were solved numerically using the COMSOL Multiphysics 3.5a solver, based on the finite-element method (COMSOL 2008). This tool has previously been applied to, *e.g.*, model flow at the pore scale (Keller and Auset 2007; Bradford and Torkzaban 2008; Torkzaban *et al.* 2008b; King *et al.* 2009), but it can also be applied to solve field scale equations (Li *et al.* 2009). Clearly, there are a wide range of computer tools that can be used to solve subsurface flow and transport equations. COMSOL Multiphysics has an advantage when its flexibility is considered with regards to the geometry, the boundary conditions, as well as the various flow, transport, and reaction processes that could be accounted for.

Illustrating the findings in simplified field scenario (Paper II)

In *Paper II*, a predictive model was constructed to exemplify the findings in the review, and to evaluate the removal efficiency of a simple sand filter system: an unplanted, vertical flow constructed wetland. The methodology includes the design and application of a two-dimensional model of flow, transport and attenuation. In *Paper II*, Λ_{fate} was defined according to:

$$\Lambda_{fate} = -\theta_W \frac{\partial C_{Wim}}{\partial t} + \frac{\partial C_{ina}}{\partial t} = -\theta_W (k_{ret} + k_{ina}) C_{Wm} \tag{14}$$

where C_{ina} is the concentration of inactivated bacteria [NL^{-3}], k_{ret} [T^{-1}] is the retention rate and k_{ina} [T^{-1}] is the inactivation rate. In addition, the effect of instantaneous retention was addressed and a Freundlich isotherm was applied (*e.g.*, Matthess *et al.* (1988)):

$$\frac{\partial C_{Wim}}{\partial t} = \frac{\partial K_d C_W^{m_f}}{\partial t} \tag{15}$$

where K_d [L^3M^{-1}] and m_f are the Freundlich coefficients that apply to a certain combination of filter media, colloids and temperature. Other isotherms, such as Langmuir (Steenhuis *et al.* 2006) and linear isotherms (Tufenkji 2007) could also have been employed to describe the microbial transport. The bacterial reaction parameters were derived from a previous lysimeter study in the unsaturated zone (Mosaddeghi *et al.* 2010).

The geometry modeled is depicted in figure 3. The top region contains sand and the bottom contains gravel. Infiltration was spread evenly and at a steady rate over the top surface, which, in reality, could be implemented using a network of pipes and large stones. Outflow occurred from the bottom right in the gravel region (to implement this in reality, tile drains could be used). The filter depth was 1 m, in agreement with Danish guidelines, and the top area was 2x5 meters, *i.e.*, two person equivalents according to Austrian guidelines (Brix and Arias 2005; Langergraber and Simunek 2005). As the domain was homogeneous in width, only a two dimensional geometry (side view) was modeled.

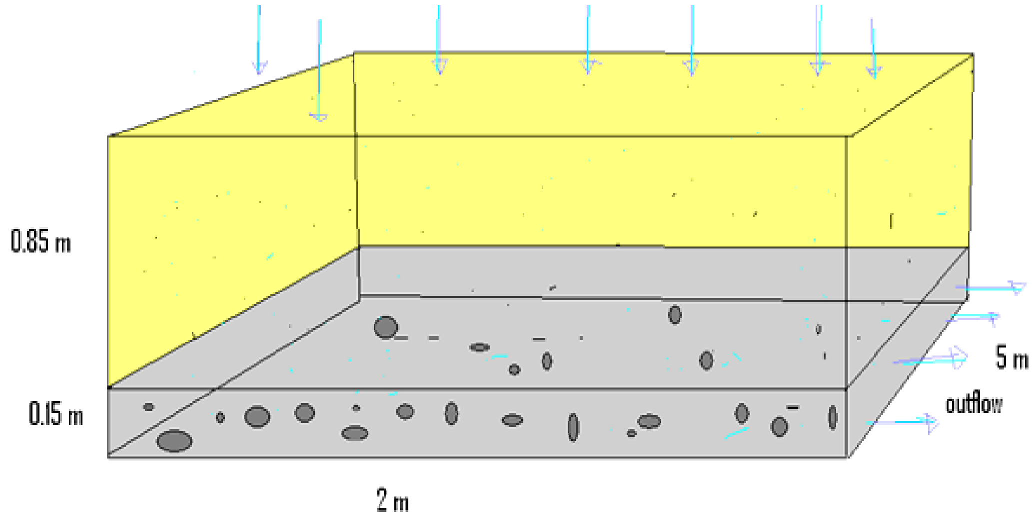

Fig. 3. Conceptual sketch of the simulated unplanted, vertical flow constructed wetland (not to scale). The upper region (yellow) contains fine grained sand and the bottom layer (gray) contains gravel. Inflow only occurs from the top and outflow only occurs at the bottom right; the remaining boundaries are assumed to be impermeable.

Predictive modeling on the basis of literature results (Paper III)

In *Paper III*, *E. coli* transport and fate in a simple filter was evaluated by applying six different literature models. The filter medium was sand, with van Genuchten parameters according to Carsel and Parrish (1988). These parameters are listed in Table 1, *Paper III*, which summarizes the default model inputs. In the simulations, the transport was calculated according to a one-dimensional version of eq. 5, in which C_W was replaced by C_{Wm} [NL^{-3}], which is more precisely defined as the concentration of mobile bacteria (*i.e.*, those that are transported with the fluid). The impact of molecular diffusion was ignored, which is common practice (Toride *et al.* 2003). The dispersivity was estimated using an α_L to distance ratio of 0.06, as reported for saturated conditions by Pang *et al.* (2004). Bacterial fate, Λ_{fate} in eq. 5, was described using four different model structures, obtained from previous studies. Primarily, Mosadegghi *et al.* (2010) applied a first-order, irreversible spatial removal equation to describe attenuation (Model 1). According to Pang (2009), the temporal removal rate can be obtained by multiplying the spatial removal rate by the pore velocity, and the removal process could then be modeled according to:

$$\Lambda_{fate} = -\theta_W k_{rem} C_{Wm} \tag{7}$$

where k_{rem} [T^{-1}] is the temporal removal rate. Further, Chen (2008) assumed deposition to be a kinetic and reversible process. These processes can be described by:

$$\Lambda_{fate} = -\theta_W k_{dep} C_{Wm} + \theta_W k_{rel} C_{Wim} \tag{8}$$

where k_{dep} [T^{-1}] and k_{rel} [T^{-1}] are the deposition rate to, and the release rate from, the immobile phase, respectively; and C_{Wim} [NL^{-3}] is the number of *immobile* cells per volume fluid, described by:

$$\frac{\partial \theta_W C_{Wim}}{\partial t} = \theta_W k_{dep} C_{Wm} - \theta_W k_{rel} C_{Wim} \tag{9}$$

Alternatively, Powelson and Mills (2001) assumed that a part of the bacteria was irreversibly removed from the mobile fluid phase, and that a part was subject to a linear equilibrium isotherm, *i.e.*, they were instantaneously adsorbed and retarded as compared to the fluid, according to Toride *et al.* (1995):

$$\Lambda_{fate} = -\rho_b K_d \frac{\partial C_{Wm}}{\partial t} - \theta_W k_{rem} C_{Wm} \tag{10}$$

where K_d [L^3M^{-1}] is an empirical distribution coefficient and ρ_b [ML^{-3}] is the bulk density of the filter media. The retardation factor, *i.e.*, the reduction in the velocity of bacteria as compared to the water flow, is calculated according to: $R = 1 + \frac{\rho_b}{\theta_W} K_d$. Lastly, Jiang *et al.* (2007) applied a model that included the combination of a reversible solid-water interface deposition site with an equilibrium isotherm:

$$\Lambda_{fate} = -\rho_b K_d \frac{\partial C_{Wm}}{\partial t} - \theta_W k_{dep} C_{Wm} + \theta_W k_{rel} C_{Wim} \tag{11}$$

Bacteria retained at the solid-water interface were modeled according to:

$$\frac{\partial \theta_W C_{Wim}}{\partial t} = \theta_W k_{dep} C_{Wm} - \theta_W k_{rel} C_{Wim} \tag{12}$$

The breakthrough curves were simulated for the models above and coefficients derived from the literature (Table 1 in *Paper III*), for various saturations (20 %, 40 %, 60 %, 80 % and 99 %) and filter depths (25 cm and 50 cm). These models were included since all of the corresponding, referred experiments evaluated the steady-state transport of *E. coli* in unsaturated porous media of approximately the same grain size and column length; no additional, similar transport studies could be found. The filter depth of 25 cm was chosen as this corresponded fairly well with the experimental conditions in the cited studies, and the depth of 50 cm was evaluated since it is a common sand filter depth. In all studies, the growth was considered to be negligible, in accordance with the cited experimental studies. The geometry was one-dimensional and the infiltration occurred at a steady rate at the top of the sand filter. Downward flow occurred due to gravity. Initially, the concentration in the domain was zero and the influent contained no bacteria. The influent concentration was set to increase suddenly (after 1h) to 1e6 CFU /100 ml. The fraction of transported cells, C_{frac}, was calculated according to (Pang *et al.* 2004):

$$C_{frac} = \frac{\int_0^\infty Q(t) * c(t)\, dt}{\int_{istart}^{iend} Q(t) * c_0(t)\, dt} x\ 100\% \tag{13}$$

where $Q(t)$ is the flow rate [L^3/T], c_0 is the inflow concentration [N/L^3] and $istart$ [T] and $iend$ [T] are the start and the end time of the pulse, respectively. The peak relative effluent is the maximum concentration in the effluent as compared to the influent, here denoted as P_{rel}.

RESULTS

This chapter presents the most important results in the literature review and in the quantitative studies.

Transport and fate of *E. coli* - key findings in the review (*Paper I*)

This section outlines the key results from the literature review as related to the underlying removal processes, the modeling approaches, as well as the key influencing factors.

Underlying processes

In order to improve an understanding of the main factors controlling colloidal transport, deposition and subsistence, the most common approach has involved bench-scale column studies, where the concentration of the effluent microbial concentration is evaluated at a certain point as a function of time (Tufenkji 2007). However, in such studies, only integrated results are observed. The key *E. coli* attenuation processes in unsaturated sand are, hence, still under debate. Important recent results are outlined below.

Retention In this thesis the distinction is made between attachment, *i.e.*, physicochemical deposition or adsorption at a single interface, and straining, *i.e.*, retention due to multiple interfaces. This is in agreement with Bradford and Torkzaban (2008), who reported that different torques and forces (energies) acted on colloids that were attached vs. those which were strained. The terms deposition and retention relate to both mechanisms. A multitude of retention processes have been proposed (Fig. 4): attachment to the solid-water interface; attachment to the air-water interface; wedging at the grain-grain contact point (straining); bridging between already deposited bacteria (straining); retention at the soil-air water meniscus (straining); retention due to bacterial rotation or stagnation and subsequent entrapment, typically near the solid-air water interface (straining); and film straining in the thin water film around a grain (straining).

Considering a simplified, natural scenario: a transporting solution of low ionic strength, negatively charged filter media (*e.g.* sand) and homogeneous, steady-state flow, literature findings, however, suggest that attachment to air or soil interfaces is limited, since their negative charges imply repulsive energy barriers (section II.D, *Paper I*). Considering recent pore-scale imaging and column studies, the main retention processes for a motile *E. coli* in the unsaturated subsurface (steady-state flow) is, instead, likely to be as follows. At first, assuming that it is transported in a network of thick and connected pendular rings, it will be weakly (reversibly) attached, due to repulsive electrostatic forces, at some distance (about 40 nm) to the soil grains (section II.B.4 and II.D.2 in *Paper I*). Findings further indicate that it will subsequently swim, or be translated, near to the grains, due to hydrodynamic forces, until it encounters a low velocity region, likely to be placed near the solid-air-water contact line. Then the bacterium becomes trapped, due to, *e.g.*, stagnant flow, capillary pinning forces, or attractive cell-collector interactions at close distances (section III.B in *Paper I*). The *E. coli* is retained. If it is transported in a region of low saturation, with thin pendular rings, it might be retained at directly the solid-air-water interface, without the preceding, weak attachment. This hypothesis is consistent with what has been reported by McClaine and Ford (2002a; 2002b), Vigeant *et al.* (2002) and Torkzaban *et al.* (2008b), as well as by the thermodynamic calculations by Chen (2008), and pore-scale visualizations of abiotic colloids of similar surface charge and hydrophily as *E. coli* by Steenhuis *et al.* (2006), Zevi *et al.* (2006; 2005) and Crist *et al.* (2004; 2005). It is also coherent with the theory that straining - as compared to attachment - is more significant than that which has been previously assumed, an idea promoted by Bradford *et al.* (2004; 2003; 2006). It is, however, possible that the deposition behavior varies with the type of *E. coli* strain. The importance of weak attachment is, for example, likely to vary with the bacterial motility and charge.

Survival In general only a small amount of data exists on microbial activity in the natural subsurface, due to high field complexities; thus, the understanding of growth and survival processes of indicator bacteria in subsurface environments is limited (Taylor *et al.* 2004). In column studies, growth is, additionally, often considered to be negligible due to the low temperature or short removal time and lack of nutrients (Jiang *et al.* 2007; Chen 2008; Chen *et al.* 2010). As *E. coli* is an enteric bacterium, inactivation is most likely to be the main subsistence process in soil (Foppen and Schijven 2006). Microbial inactivation mechanisms include natural die-off and predation by nematodes and protists, *e.g.*, protozoa (Jamieson *et al.* 2002; Keller and Auset 2007; Wand *et al.* 2007; García *et al.* 2010). Bacterial subsistence in biofilm is discussed below. The interdependence of a microorganism's growth and response to nutrient availability and survival stress was underlined by Ginn *et al.* (2002): survival mechanisms are linked to active adhesion and detachment, as well as chemotaxis, *i.e.*, the ability to move in response to a chemical gradient, such as nutrient availability (Wang and Ford 2009). Chemotaxis could cause faster bacterial transport (Wang and Ford 2009). Moreover, microbes can attach onto, and be co-transported with, organic colloids, *e.g.*, to areas of higher nutrient content – a process that can protect them from inactivation (Pang 2009).

Biofilm, bioclogging and cell aggregation The mechanisms proposed above do not account for bacterial behavior in the presence of biofilms, *i.e.*, surface-associated multicellular communities of microorganisms, embedded in a matrix of extracellular polymeric substances (Branda *et al.* 2005; Strathmann *et al.* 2007). It is probable that their presence substantially affect *E. coli* retention and subsistence. They are even thought to be responsible for the majority of the microbial processing that occurs in subsurface constructed wetlands (García *et al.* 2010). Biofilms could protect the bacteria from environmental stresses (Hall-Stoodley *et al.* 2004) and serve as reservoirs (Lamb *et al.* 1998). Moreover, as *E. coli* in unsaturated media in the field often originate from septic tank systems or manure application, it is probable that other types of microbes will be present (Wang *et al.* 2011). These can produce biofilms and change the filter media surface characteristics, which could affect the *E. coli* transport and retention behavior. The biofilms could increase the attachment rate, as they have sticky properties and could provide attachment sites at extracellular polymeric substances, cell walls and lipid membranes, and the cytoplasm (Strathmann *et al.* 2007). It is also possible that bioclogging influences the hydrodynamic properties of porous media through increased surface roughness, reduced porosity and permeability, or preferential channeling that could increase the conductivity locally (Liu and Li 2008; Bauman *et al.* 2009). Hence, the presence of biofilms could also increase the straining rate, since pathways become narrower. Additionally, biofilms could influence the detachment behavior: Wang *et al.* (2011), *e.g.*, reported that biofilm sloughing and erosion had probably contributed to the release of *E. coli*. These processes become pertinent if there are sudden changes in the flow rate. Lastly, the survival rate might be affected, as the bacteria could be trapped in biofilm and then be grazed upon by protozoa (Bauman *et al.* 2009; García *et al.* 2010). However, column studies on the effect of a biofilm and extracellular structures on bacterial transport are relatively rare and inconclusive, especially in unsaturated soil. Biofilms have been reported to both hinder microbial transport and to not affect it significantly (Chabaud *et al.* 2006; Tufenkji 2007; Liu and Li 2008; Salvucci *et al.* 2009). Bacterial biofilm development is more important in systems of high moisture content (Dechesne *et al.* 2010).

In addition, bacteria can aggregate in the fluid (in so called flocs), or when they are retained on a surface (Sirivithayapakorn and Keller 2003; Kim *et al.* 2009). Flocs affect straining due to the larger size of the transported unit (Stevik *et al.* 1999a; Schinner *et al.* 2009). However, if particles are diluted, as is likely to apply in natural environments, it has been argued that the probability of a collision between two cells is minimal (Chen and Zhu 2005). Further, pore-scale imaging in the laboratory has revealed that hydrophilic microspheres often adhere to already deposited microspheres (Zevi *et al.* 2005; Zhang *et al.* 2010). Zhang *et al.* (2010) reported that colloids, once retained, acted as new deposition sites for other suspended colloids and that the deposition rates were dependent on the input concentration; however, clogging and aggregation are more important in the cases of high input particle concentrations (Zhang *et al.* 2010). The implication in the vadose, a few decimeters from the ground surface (with low input concentrations), is that the importance of biofilm development, aggregation and clogging is likely to be limited.

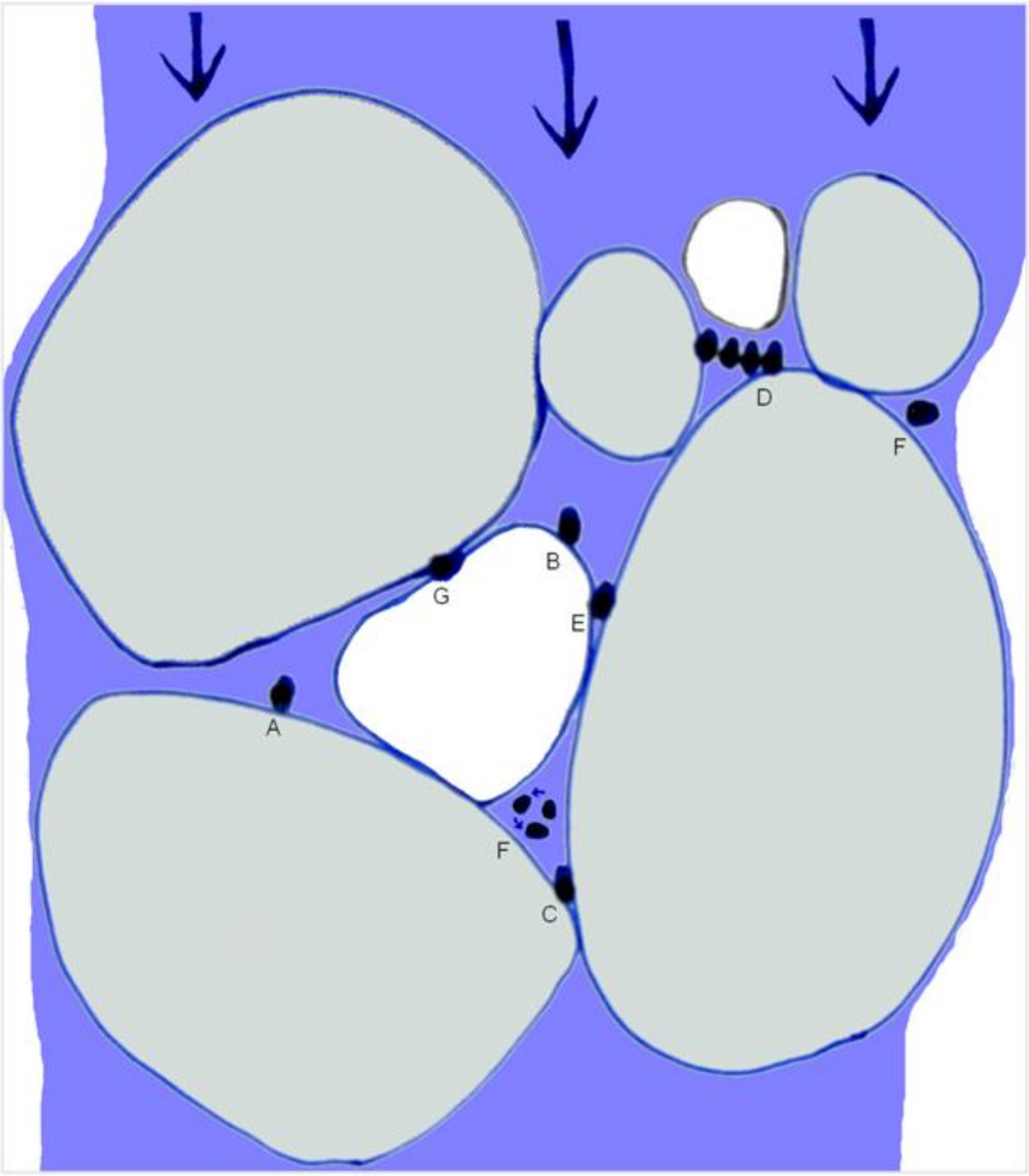

Fig. 4. Conceptual sketch of colloidal retention mechanisms proposed in unsaturated media: A) attachment to the solid-water interface; B) attachment to the air-water interface; C) wedging at the grain-grain contact point; D) bridging between already deposited bacteria; E) retention at the soil-air water meniscus; F) retention due to bacterial rotation or stagnation (and subsequent entrapment), e.g., near the solid-air water interface; and G) film straining in the thin water film around a grain. The figure is redrawn from Bradford and Torkzaban (2008).

Influencing factors

Findings in *Paper I* point to the fact that physical factors, such as flow rate, moisture content and temperature have the largest impact on the removal of *E. coli* in dynamic systems. Table 2 in *Paper I* lists the various factors that are hypothesized to influence *E. coli* retention in unsaturated dynamic systems, sorted by the spatial first-order total removal rate, k_{rems} [L^{-1}]. The findings based on this table indicate that the flow rate is a determinant factor for *E.*

coli retention in solutions of low ionic strength. The relative effluent concentration is, for example, very low (< 0.1 %) in the majority of the cases with an average Darcy velocity, q, of less than 0.1 cm/min. The correlation coefficients between the spatial removal rate and the Darcian fluid flux (q), saturation, grain size, as well the solution ionic strength were calculated. However, among these factors, only the correlation between $log(q)$ and $log(k_{rems})$ was significant (p-value = 0.001) (Fig. 5). The slope was -1.2, which means that $k_{rem} \propto 1/q^{1.2}$, and this underlines the importance of the flow rate as a key influencing factor. In a coherent manner, Pang (2009), reported that pore-water velocity showed the clearest correlation with spatial removal rates in aquifers for all the evaluated properties (pore-velocity, distance, porosity, particle size). She further argued that this result was consistent with findings for unsaturated zones. Moreover, Stevik *et al.* (1999a) reported that it was likely that physical factors were more important than chemical factors (pH, cation exchange capacity and ionic strength) for *E. coli* removal under unsaturated conditions–a result which was attributed to the great importance of fluid shear forces. Nonetheless, the flow rate is also reduced by a lower moisture content and grain size (Lazouskaya *et al.* 2006; Jiang *et al.* 2007). Hence, these factors might affect the attenuation efficiencies indirectly. In addition, increased ionic strength could augment weak attachment and funneling to low-velocity regions. The importance of physical factors on retention supports the idea that solid-air water interface straining is an important retention process: reduced flow velocity reduces the drag force near the solid-air water wedges that trap the bacteria and, hence, increase the retention; reduced water content reduces the size of the wedges and the occurrence of locally saturated regions; and reduced filter media size and increased surface area increases the capillary forces and reduces the size of the solid-air-water wedges. It should, however, be noted that studies in relation to the importance of biological factors on *E. coli* transport in unsaturated media are rare.

Survival As regards inactivation, nutrient and moisture content have a critical influence. Naturally, the presence of nutrients increases survival. In saturated media, the temperature has further been reported to be a key influencing factor: its increase generally increases inactivation (Foppen and Schijven 2006). Furthermore, experimental findings suggest that the moisture content is a key influencing factor for biofilm growth for motile bacteria (Or *et al.* 2007; Dechesne *et al.* 2010; Wang and Or 2010; Wang *et al.* 2011), such as *E. coli*. The saturation moreover affects the development of biofilms of other microbes, and these can host predators, which could have a significant impact on *E. coli* inactivation and increase straining. Or *et al.* (2007) emphasized the special nature of the unsaturated subsurface habitat: it is diverse, localized, and contains small bacterial colonies that are situated near the subsurface interfaces. The habitats are often formed in the solid-liquid-gas wedges (Or *et al.* 2007), which are connected by means of thin water films. Mobility is essential for colony expansion, and dispersal by flagellar

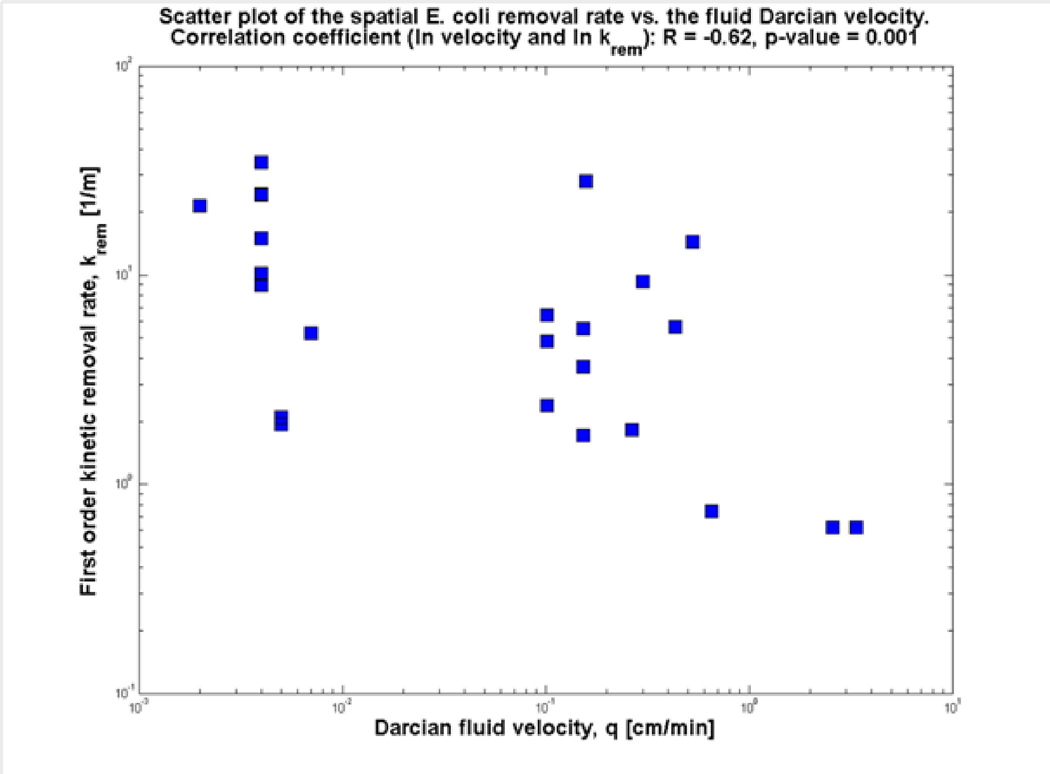

Fig. 5. Correlation between the spatial E. coli first-order attenuation rate, k_{rems} [1/m], and the Darcian flux (data from Table 2, Paper I). The correlation coefficient R of the log velocity and log k_{rems} is - 0.62, the p-value is 0.001 and the slope is -1.2.

motility is only possible within a narrow range of wet conditions (Dechesne *et al.* 2010). Nevertheless, knowledge relating to bacterial biofilm development and its impact on removal in dynamic, unsaturated systems is, as yet, limited (Wang and Or 2010).

Modeling approaches

The means by which the influencing factors, discussed above, could be incorporated in predictive models has not yet been established. The commonly applied theory for saturated transport, the colloid filtration theory (Yao *et al.* 1971; Tufenkji and Elimelech 2004), is likely not to be relevant for the unsaturated transport of *E. coli*, as it does not account for the presence of air in a system, and it assumes that retention only occurs due to favorable attachment to filter media grains (section II.B, *Paper I*). A multitude of processes have been proposed to account for *E. coli* removal. Models have, for example, accounted for: reversible (Chen 2008) or irreversible processes (Schäfer *et al.* 1998); one deposition mechanism (Pang 2009) or multiple (Foppen *et al.* 2007); time- or depth dependent retention (Bradford *et al.* 2003); a maximum attainable deposited concentration (Ko and Elimelech 2000); distinct solid-water and air-water interface retention processes (Lenhart and Saiers 2002), or in combination (Gargiulo *et al.* 2008); and distinct attachment and straining processes (Simunek *et al.* 2006), or in combination (Corapcioglu and Choi 1996). A similar diversity has also been discovered for *E. coli* in unsaturated media, specifically (Table 2, *Paper I*). The proposed mechanisms are sometimes conflicting, despite of the fact that the experimental scenarios are often quite similar. The modeling approaches are generally fitted to integrated results (breakthrough curves); thus, the model coefficients could represent multiple, alternative, deposition processes (DeNovio *et al.* 2004; Johnson *et al.* 2010). Smith *et al.* (2008) state: "*with any optimized modeling investigation involving several parameters, similarly good fits can be achieved with more than one combination of variables*". The idea that different underlying processes could explain the same integrated outcome has been named equifinality in the context of hydrological modeling (*e.g.*, Beven and Freer (2001)). In summation, the underlying attenuation processes are not, as yet, fully known, and there is no generally accepted model structure by which *E. coli* transport could be predicted. This also applies to comparatively simple contexts, such as steady-state conditions and transport in homogeneous soil in a laboratory. Considering the limited current knowledge, a pragmatic modeling approach in the field is the kinetic first-order irreversible model, with all removal mechanisms lumped into one coefficient:

$$\Lambda_{fate} = -\theta_W k_{rem} c_{Wm} \qquad (16)$$

where Λ_{fate} is the bacterial fate term in eq. 5 and k_{rem} [T^{-1}] is the removal rate. Studying microbial transport and removal under both saturated and unsaturated conditions, Pang (2009) reported that 61 out of 87 studies were satisfactorily described using such a model. This approach was also applied to *E. coli* in unsaturated, undisturbed field columns by Mosaddeghi *et al.* (2009; 2010) as well as Unc and Goss (2003). Clearly, alternative models to this basic approach are possible; however, it is preferable to use such models with care in relation to the risk of over-parameterization.

Pore-scale imaging Improved understanding of the underlying processes could be made possible through imaging at the pore scale (Smith *et al.* 2008). One method for studying various pore-scale phenomena is to dye the particles, the filter media and the transporting solution, and to use image processing techniques to count the number of particles (black pixels) at a few, different types of interfaces (Steenhuis *et al.* 2006). However, it is a challenge to relate such pore-scale observations with Darcy-scale systems (Smith *et al.* 2008). Theoretical approaches in relation to handling the translation between length scales are under development. This is further discussed below.

Illustrative modeling (*Paper II*)

The results for the simulation of the vertical flow constructed wetland are presented in this section. Figure 6 displays the velocity field (arrows and streamlines) as well as the concentration (surface) after 1 week (kinetic retention). The flow velocity is, as expected, higher in the bottom region: the average velocity is 0.4 cm/h and 2.5 cm/h in the unsaturated sand (69 %saturation) and saturated gravel (100% saturation), respectively. The influent concentration was 1.2e6 CFU/100 ml and the average effluent concentration was 2.1e4 CFU/100 ml; hence, the total log10 removal was ≈ 1.8. In figure 3 in *Paper II*, the breakthrough curves at different depths are plotted over time. Removal mainly takes place in the unsaturated zone, which was expected considering the higher retention rate and lower flow velocity in this region. After 1 week, the effluent concentration (at the outlet boundary) stabilized at 2.1e4 CFU/100 ml (Fig. 4, *Paper II*).

Forward sensitivity analysis

The effect of the infiltration rate on the relative effluent concentration is shown in Table 3, *Paper II*. In this case a forward sensitivity analysis removal was considered to be a kinetic process and the coefficients were the same as in the previous section. Clearly, inward flux has a large impact on removal: *e.g.*, for an infiltration rate of 0.04 cm/h, it would take approximately 200 days for the bacteria to reach the outlet (assuming they travel with the water). After that time, it is likely that the majority of the bacteria have been inactivated. The impact of various kinetic retention rates in unsaturated sand can be seen in Table 4, *Paper II* (the infiltration rate is set at 0.4 cm/h). For very high retention rates (7.85/day), no *E. coli* remains in the effluent.

Kinetic vs. equilibrium retention

When using an equilibrium model to describe retention in the unsaturated sand (Freundlich isotherm), and laboratory coefficients fitted by Jiang *et al.* (2007), the effluent concentration was calculated to be 7.6e5 CFU/100 ml. Thus, the removal was only 0.2 log10. However, inferences to be drawn from these results are limited, due to the low degree of fit of the experimental breakthrough curves to the Freundlich model (0.58) (Jiang *et al.* 2007). Additionally, no other studies on *E. coli* transport in the unsaturated zone

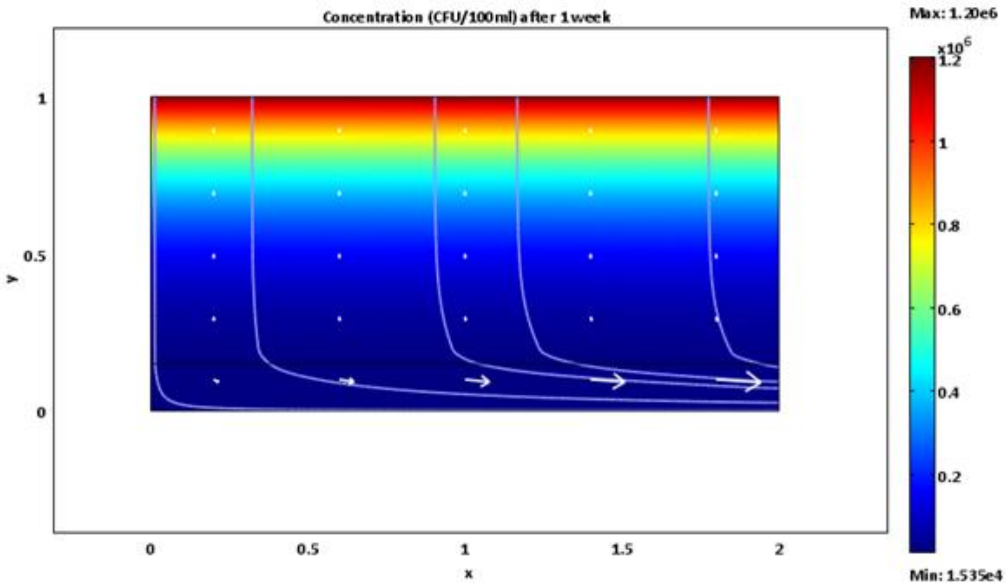

Fig. 6. Concentration (in CFU/ 100 ml) of E. coli (surface) and velocity field (arrows and streamlines) after 1 week (kinetic retention). The log10 removal in the filter was approximately 1.8.

that apply Freundlich isotherms have been found; hence, it is difficult to confirm the relevance of this result.

Predicting the efficiency of a filter on the basis of literature models (*Paper III*)

The relative BTCs are displayed in Fig. 7 and Fig. 8, considering transport distances of 25 cm and 50 cm, respectively (Table 1 in *Paper III*). Clearly, the shapes and heights of the breakthrough curves vary considerably between the different models. The values of C_{frac} and P_{rel} for each model, as dependent on saturation, are listed in *Paper III* (Appendix A). This also displays the minimum and maximum values of C_{frac} and P_{rel}. For example, C_{frac} ranges from 2.5e-9 to 1 (Θ = 40 %) and from 9.5e-4 to 1 (Θ = 80 %), and P_{rel} ranges from 4.5e-10 to 0.18 (Θ = 40 %) and from 1.9e-5 to 0.15 (Θ = 80 %). These wide ranges are remarkable considering that all of the applied models have been derived from similar experimental settings (Table 3, *Paper III*). Model 6 is notable based on its high removal efficiency results. If this model were to be excluded from the analysis, the ranges of C_{frac} would, more reasonably, be more narrow: 0.42 to 1 (at 40 % saturation) and 0.92 to 1 (Θ = 80 %), and P_{rel} would range from 0.09 to 0.18 (Θ = 40 %), and from 0.05 to 0.15 (Θ = 80 %). The breakthrough curves for the various models display different behaviors with regard to the flow velocity. The log10-reductions of the bacterial concentrations are shown for the filter depths of 25 cm (Fig. 9) and 50 cm (Fig. 10). The latter is a typical sand filter format. The darker, dashed line and the lighter dashed line show the reduction necessary for compliance with bathing and drinking water guidelines, respectively.

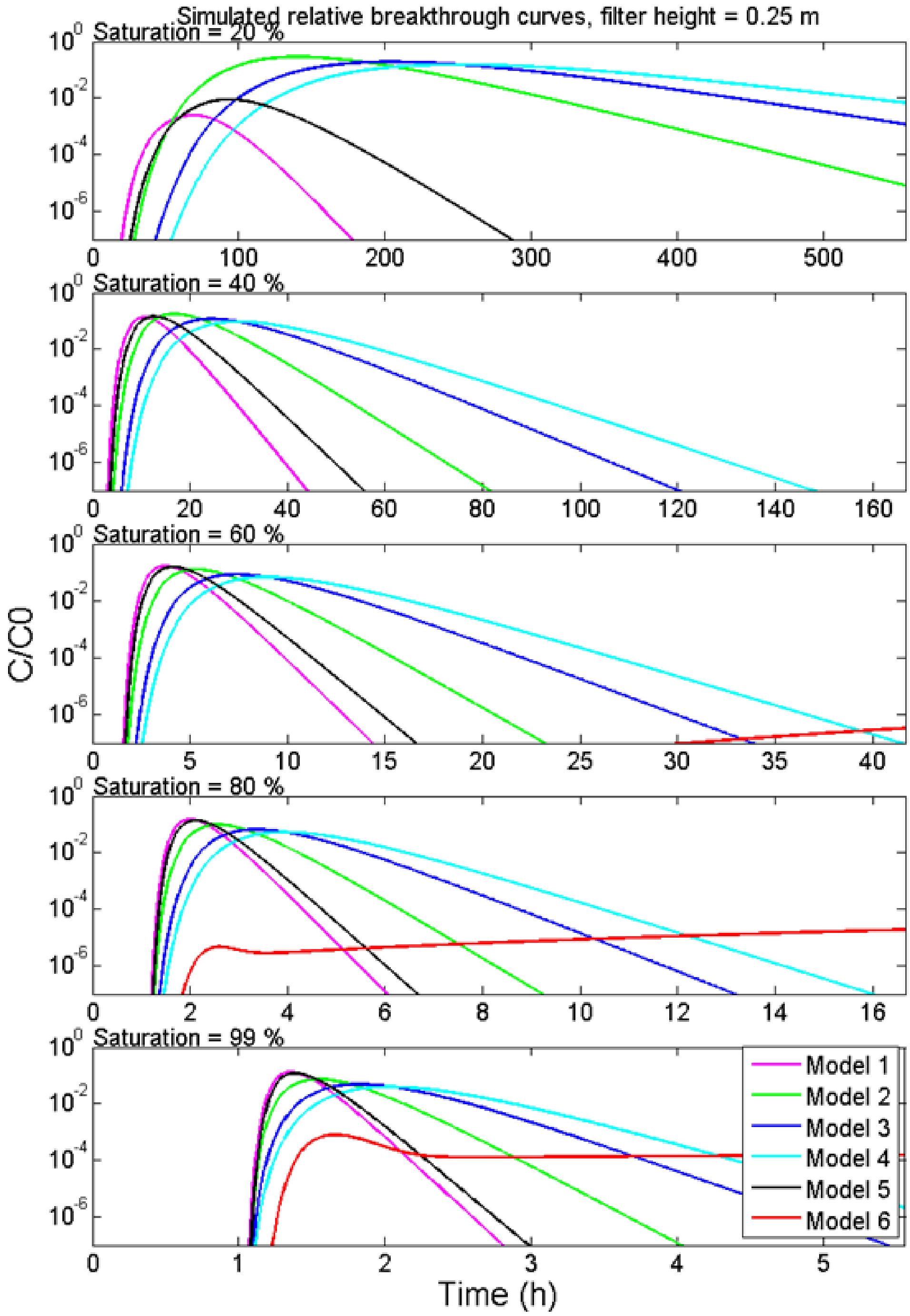

Fig. 7. Relative BTCs (C/C0) for the various models in Table 3, Paper III, considering a filter depth of 25 cm and saturations of 20 %, 40 %, 60 %, 80 % and 99 %, respectively.

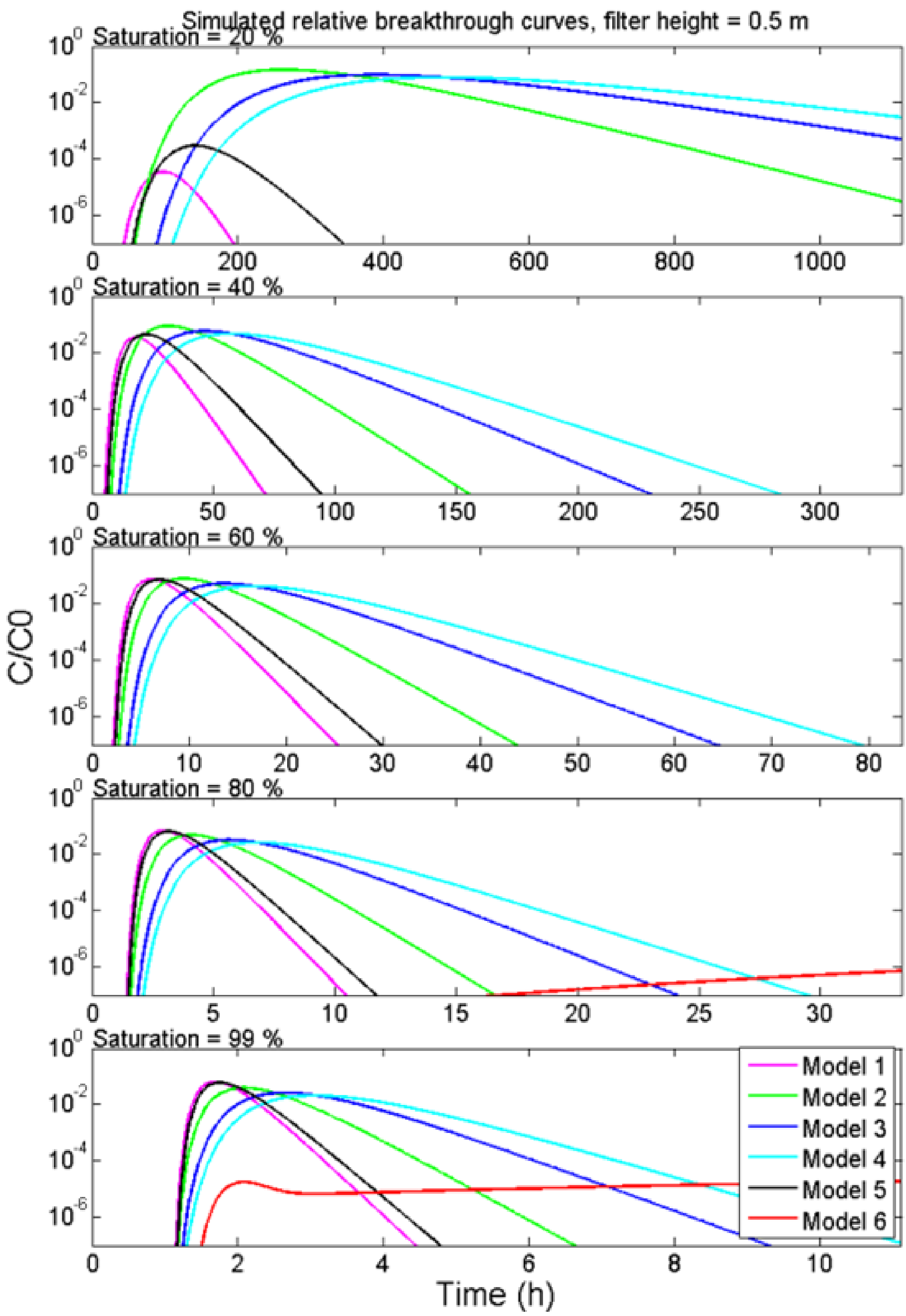

Fig. 8. Relative BTCs (C/C0) for the various models in Table 3, Paper III, considering a filter depth of 50 cm and saturations of 20 %, 40 %, 60 %, 80 % and 99 %, respectively.

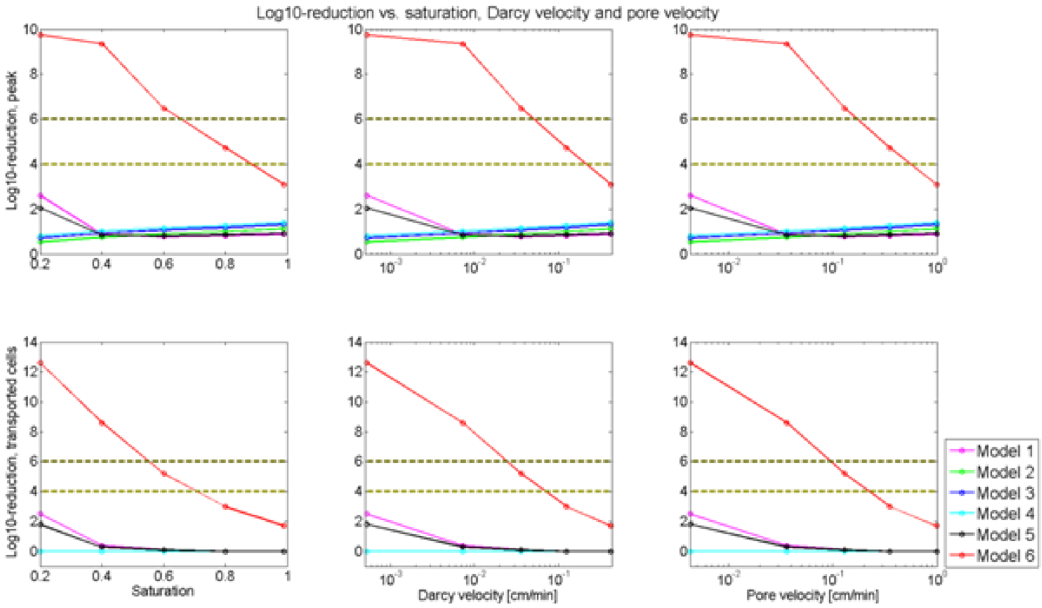

Fig. 9. The log10-reduction of the relative peak effluent concentration and the relative accumulated effluent number of cells, as related to saturation, Darcy velocity and pore velocity (filter depth = 25 cm). The darker and lighter dashed lines show the reduction necessary for compliance with drinking and bathing water guidelines, respectively.

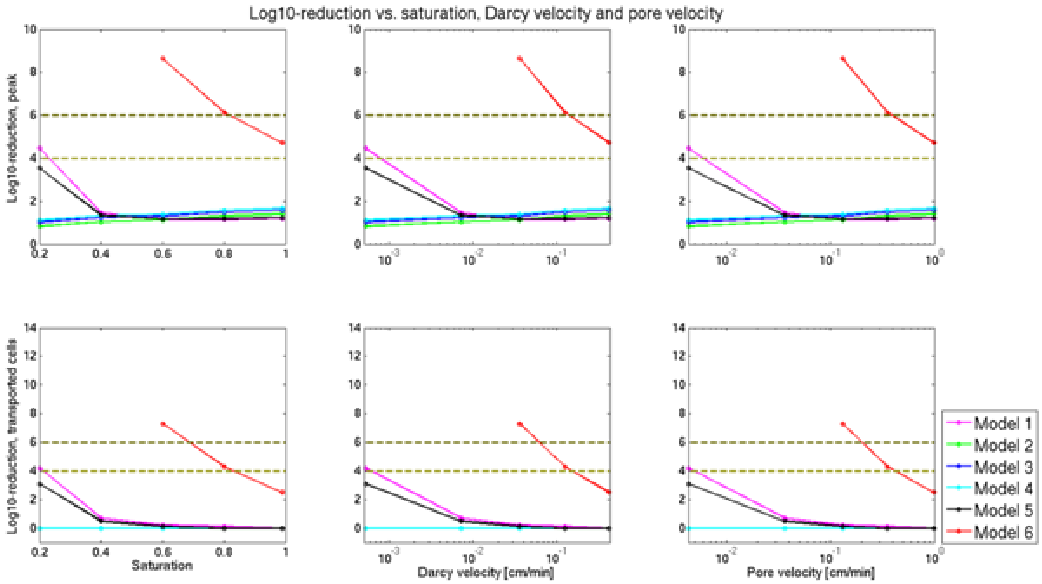

Fig. 10. The log10-reduction of the relative peak effluent concentration and the relative accumulated effluent number of cells, as related to saturation, Darcy velocity and pore velocity (filter depth = 50 cm). The darker and lighter dashed lines show the reduction necessary for compliance with drinking and bathing water guidelines, respectively.

DISCUSSION

This chapter evaluates the implications of the results presented in the previous chapter.

Generalizing previous research?

Paper I raised some important questions. For, example: can literature results be used to predict *E. coli* removal in the unsaturated zone in the field, for example in Bwaise III Parish, or even, in a simple sand filter? Could the implications of a conservative approach be outlined? Could one specific model and a range of applicable coefficients be defined for a simple, well-defined system? These questions were addressed in *Paper III*.

Explaining the wide ranges of breakthrough curve behavior? (Paper III)

In this section, the simulated breakthrough curves (Fig. 7 and Fig. 8) are analyzed in order to assess the implications of the different model structures, as well as the possibility to predict removal in the various fictitious filters. The wide ranges in breakthrough curve behavior indicate that a model can only be applied to a filter with the same saturation as that which applied in the experiment from which it has been derived (in the simulations the coefficients were specified to be independent of saturation). If the wide range was only caused by this extrapolation, then P_{rel} would, however, be similar in different studies with the same saturation (if the other experiential settings were comparable). Table 4, *Paper III* lists experimentally derived values for P_{rel} in different studies of similar saturation, filter media and depth. From this table it is evident that there is no coherency to the values. Hence, the wide ranges are, at least to some degree, related to other factors. One possible explanation is that the removal rates are considerably dependent on the van Genuchten parameters of the filter media and that these varied largely between the cited experiments – and were different from those reported in Carsel and Parrish (1988) (applied in *Paper III*). This would limit any generalizations of bacterial removal to filters involving the same van Genuchten parameters, and underline the importance of publishing these parameters for each studied filter, which is not always the case at the present time. Another explanation is that the type of *E. coli* strains varied in the experimental studies, and that the type of strain has a large influence on the retention behavior.

Clearly, this is a complicated issue. The assumption that the temporal coefficients are perfectly independent of the saturation (and flow rate) is likely to be a simplification. Nevertheless, *Paper III* aims at illustrating how one could make use of published results in order to specify a *range* of realistic outcomes in systems of different settings, as well as to specify a conservative approach. It provides a methodology to compare previous research. Other strategies to generalize literature results have not been found. Alternatively, predictions would only be based on very few models; however, this would probably make them less robust. Therefore, this simplification was accepted.

The implications of the various model structures (Paper III)

Models 1 and Model 5 result in similar BTCs apart from the slight delay and reduced slope of the tail of the latter, which has occurred due to its equilibrium isotherm. This means that a part of the bacteria deposits instantaneously. It moves slower than the fluid. However, many studies have shown that, contrarily, colloids often sample *faster* (wider) pathways than tracers (size exclusion), which occurs due to the significant size of the bacterial particles (1 μm) (Unc and Goss 2003; Keller and Auset 2007; Foppen *et al.* 2008; Mosaddeghi *et al.* 2010;). Hence, the probability is that their transport will, in general, be *accelerated* as compared to water. A conservative approach, therefore, does not include an equilibrium isotherm (Pang *et al.* 2004). Moreover, instantaneous deposition means that the bacteria would instantaneously be released if the bacterial concentration in the fluid were to be zero; however, a recent study by Torkzaban *et al.* (2008b) reported that only a small fraction of the retained *E. coli* was released when the column was flushed with clean water.

One important difference between the models further relates to whether the kinetic deposition is considered reversible (models 2, 3, 4 and 6) or irreversible (models 1 and 5). Reversible processes entail that bacteria are loosely retained and result in long tails of the BTC (Fig. 7 and Fig. 8). A high deposition rate results in a BTC peak that is late, short and wide, whereas a high release rate causes the reverse: it is early, high and thin (Jiang *et al.* 2007). In models 2-4 C_{frac} is 100 %, which is expected, since both the deposition and release coefficients are very high and the retained cells are not inactivated: all bacteria are quickly released from deposition. If a reversible model is assumed, it is recommended that a decay term is added for the retained bacteria; otherwise, if the model is applied in a setting involving a different flow rate or transport distance, the result could be that C_{frac} equals 100 %, which might not be realistic. For example, the experimental values of C_{frac} that could be found are clearly less than 100 %: C_{frac} equals 29 %, 63 % and 1 %, for experiments 1, 5 and 6, respectively (Table 2, *Paper III*). Further, regarding field conditions, Crane and Moore (1984) stated: "*Bacteria generally move less than 1 m when unsaturated flow conditions prevail in a soil*". This points to the fact that irreversible removal does occur in the field.

First-order removal with constant temporal coefficients (Paper I and Paper III)

Recent literature results point to the fact that kinetic irreversible removal (eq. 7) could be an acceptable approximation when the pore-scale retention processes are not known and that it is not very likely that the first-order temporal attenuation are considerably dependent on the flow velocity. These findings are summarized below. Literature results have shown that 70 % of 87 reviewed field studies relating to microbial transport could satisfactorily be described using a kinetic, irreversible attenuation model (Pang 2009). The author further stated that the deviation from this model mainly applied to organically contaminated fine filters, something that clearly does not regard the column studies cited in *Paper III*. In addition, it was reported that, of all the evaluated aquifer properties (pore-velocity, distance, porosity, particle size), the pore-water velocity, v, was the parameter that showed the clearest correlation with the spatial first-order removal rate: $k_{rems} \propto v^{-0.62}$. Pang (2009) argued that this result was consistent with findings for unsaturated zones: spatial removal rates decrease with increased hydraulic conductivity and infiltration rates. Accordingly, figure 5 above shows a significant inverse correlation coefficient between the flow velocity and the spatial first-order removal rate: the slope of the relation between $log\ q$ and $\log(k_{rems})$ was -1.2 (p-value = 0.001). The implication is: $k_{rems} \propto (v\theta_W)^{-1.2}$. These results support the idea that the temporal first-order removal rate, k_{rem}, is only slightly (inversely) correlated to the pore-water velocity, *i.e.*, $k_{rem} \propto \mathrm{v}^{-0.2}$, considering that $k_{rem} = vk_{rems}$, per definition (Pang 2009).

Evaluating the results with respect to health guidelines and field studies (Paper II and Paper III)

The US water quality criterion for fresh recreational waters is that the geometric mean of the observed bacterial density is < 126 CFU /100 ml (USEPA 2003). In a conservative approach, it is assumed that no attenuation occurs after the treated water is released from the filter. As the influent concentration is 1e6/100ml (*Paper III*), the bacterial reduction is thus required to be at least 4 log10 (or, more precisely 3.9 log10) in order to comply with the guidelines. For drinking water quality, the WHO standards are a concentration of < 1 CFU/100ml WHO 2008; hence the reduction should be 6 log10. The log-10 reduction for the various flow rates was calculated in the filters of depths of 25 cm and 50 cm (Fig. 9 and Fig. 10). The darker and the lighter dashed line show the reduction necessary for compliance with drinking and bathing water guidelines, respectively. A reasonable conservative approach is that all models should result in a sufficient reduction for a filter to comply with the guidelines. Visual inspection of the graphs, however, reveals that neither of the filters is sufficiently deep for the required reductions of P_{rel} and C_{frac}: for filter depths of 50 cm, only Model 6 (Θ < 60%) results in a sufficient reduction for drinking water quality.

Alternatively, a first-order kinetic removal model could be applied to evaluate the required filter depth (coefficients as derived in Table 2, *Paper III*). Using this methodology, the travel distances necessary for compliance with bathing and drinking water guidelines, respectively, are listed in *Paper III*, Table 6 and Table 7: at 20 % saturation, the filter depth would have to be at least 44 cm and 66 cm, respectively. However, to obtain a Darcy velocity that is more typical for sand filters: 0.004 cm/min (Falvey 1997), the saturation must be at least 35 %. At 40% saturation the filter must be at least 3.8 m and 5.7 m to comply with bathing and drinking water guidelines, respectively. For faster flows, remarkably higher filter depths

(> 10 m) are required. It is moreover, clear that saturation (or flow rate), as compared to filter depth, has a larger influence on the attenuation efficiency. This is in agreement with the findings by Pang (2009), discussed above.

In *Paper II*, the effluent concentration (2.1e4 CFU/100 ml) is two orders of magnitude larger than the guidelines for bathing water quality. This result indicates that constructed wetlands should be designed with: deeper unsaturated zones, *i.e.*, at least one more meter (than 0.85 m); lower infiltration rates (<0.4 cm/h); or finer filter media, than those specified in *Paper II*.

Regarding field transport, Crane and Moore (1984) stated that septic tank disposal systems present little hazard to groundwater contamination, under the premise that discharge occurs to unsaturated soils at least 1.5 meters above the water table. Considering the results in *Paper III*, this statement would, however, only apply to soils of low moisture contents (< 40 %) and flow rates. In the natural environment, the attenuation rates of microbes in superficial soil, which is normally considered unsaturated, is a few log10/m for the majority of soil types (Pang 2009). This is in agreement with the spatial first-order log-removal rates calculated for experiments 1-5 (corresponding to models 1-5): 1.4 log/m - 5.5 log/m (1 log10/m ≈ 2.3 log/m), listed in Table 2, *Paper III*. However, experiment 6 with a log-removal of 34.5/m is rather high. On the other hand, it is normal that values are much higher in the laboratory than in the field. Moreover, the results in *Paper II* are also consistent with Pang (2009): removal was ≈1.8 log10 for 0.85 m transport in the unsaturated zone, *i.e.*, removal was approximately 2.1 log10/m.

Translation between experimental scales

There are challenges related to predictive modeling based on models derived from laboratory conditions or limited field data; well-defined laboratory experiments are rarely relevant to natural processes, which are highly complex, due to physical, chemical and biological heterogeneities, and are, as such, difficult to imitate (Tufenkji 2007). This problem is addressed in field-scale experiments; however, generalizing such findings is difficult. The upscaling of column-scale findings to the field is linked to whether the column can be considered as being a representative element volume (Pang 2009). Regarding parameters, values reported in the natural environment are, generally, much lower than those derived from short term column experiments; microbial removal rates were, *e.g.*, reported to be 1-3 orders of magnitude times lower in the field (Pang 2009). Studying *E. coli*, specifically, Smith *et al.* (1985) reported a 2 log10 attenuation difference in disturbed versus intact cores.

Furthermore, it has not yet been established as to how to relate pore-scale observations with Darcy-scale systems (Smith *et al.* 2008). This approach, however, implies that only a limited number of interfaces can be studied at a time, which might not represent the overall systems (Zevi *et al.* 2009). Theoretical approaches to handling the translation between length scales are under development; the up-scaling from cell to biofilm scale has, for example, been developed by Wood and Whitaker (1999), and from biofilm to Darcy scale, by Golfier *et al.* (2009). Common methods involve homogenization and volume averaging, which cover means by which closure problems are handled, and the implication is that sub-pore-scale parameters and problem geometry are incorporated into the definitions of the effective parameters at the macro-scale (Wood and Whitaker 2000; Wood *et al.* 2002; Golfier *et al.* 2009). The related research is still ongoing.

Study colloids to predict *E. coli* and *E. coli* to predict pathogens?

It has been debated as to whether inorganic particles could be used to satisfactorily predict *E. coli* retention mechanisms. The difference in rheology and motility between *E. coli* and abiotic colloids, as well as the ability of bacteria to survive in biofilms, clearly question the relevance of findings referring to processes and models developed for colloids. However, these biological processes are currently insufficiently understood. It has been argued that microspheres might provide a useful surrogate for *E. coli*, if they are particularly selected, based on a similarity of size and surface properties (Passmore *et al.* 2010). Knowledge of the behavior of similar colloids in various scenarios provides a starting point for *predictions* of *E. coli* removal.

Another issue concerns the relevance of the use of *E. coli* to predict the removal of bacterial pathogens in general. *E. coli* is a suitable bacterium to study because a relatively large amount of data has been collected relating to its properties and it is easy to monitor and is a useful fecal indicator. The prediction of contaminant transport using *E. coli* models, could, however, be questioned, as *E. coli* retention rates have been shown to be heterogeneous within the strain (Foppen and Schijven 2006; Yang *et al.* 2006; Haznedaroglu *et al.* 2009; Lutterodt *et al.* 2009; Foppen *et al.* 2010). For example, Foppen *et al.* (2010) reported that the maximum breakthrough concentrations varied by two orders of magnitude in 54 different *E. coli* strains. The authors recommended that further studies are conducted to assess the relevance of *E. coli* as a fecal indicator. This also suggests that future research should focus on specific, pathogenic *E. coli* strains and not on *E. coli* in general. Clearly, the transport and fate behavior of *E. coli* do not necessarily correspond to those of pathogenic bacteria. Nevertheless, knowledge of the most important *E. coli* influencing factors, key processes, and modeling approaches under specified circumstances, could provide useful information when predicting the transport of other microbes – assuming that their characteristics, as compared to those of *E. coli*, have been well identified. Further, Pang (2009) reported that the removal rates for *E. coli*, fecal coliforms, streptococci, and enterococci were similar.

The relevance of steady-state, homogeneous flow and no biofilm formation

As transport and attenuation are strongly dependent on flow processes, the predictions relating to homogeneous, steady-state flows have limited relevance in many natural environments. Some of the related mechanisms are discussed below, with citations to in-depth studies, to which the interested reader is referred.

Transient vs. steady-state flow in sand filters and nature

Regarding the natural environment, Emelko and Tufenkji (2010) argued that the impact of extreme events, such as heavy rains, should be taken into account when developing quantitative microbial risk assessment frameworks to protect groundwater supplies. In the vadose zone, microbial transport with transient wetting fronts is an important contamination mechanism (Emelko and Tufenkji 2010; Zhuang *et al.* 2010). Transient conditions can have a significant impact on bacterial behavior, *e.g.*, release from deposition and colony expansion. Retention near the air-water interface is particularly critical under transient flow conditions, as the implication is that the deposition interface moves (Bradford and Torkzaban 2008). This is particularly significant when considering that retention at the solid-air-water interface is likely to be an important *E. coli* retention mechanism; release can occur when the interface moves (Keller and Auset 2007). Moreover, regarding survival, Dechesne *et al.* (2010) concluded that even brief and infrequent wet events enabled the bacterium Pseudomonas putida KT2440 to disperse by flagellar motility, yielding larger colonies. It is probable that this finding will also apply to motile *E. coli* strains, which move in a similar way (Dechesne *et al.* 2010). The effect of infiltration and drainage on mobilization has further been evaluated by Cheng and Saiers (2009), Bridge *et al.* (2009), Zhuang *et al.* (2009, 2010), and Shang *et al.* (2009, 2008), as well as Gao *et al.* (2006), who focused on pore-scale mechanisms.

In sand filters, transient flux is possible if the filter has an open top surface; however, closed systems are not exposed to sudden influxes. In the vadose zone, changing moisture contents mainly applies to the upper part, where the saturation varies with the time of day and rainfall rates, whereas more stable moisture levels often occur at the lower part, where infiltration changes are damped down by time and matric forces (Powelson and Mills 2001). Stevik *et al.* (1999b) reported that unsaturated, steady-state flows were established at depths of approximately 20 cm and 40 cm, when eight daily doses of 3.125 mm and 6.25 mm, respectively, were intermittently applied to columns. Further, Russo and Fiori (2008) simulated transient and equivalent steady-state partially saturated flows, accounting for observed hydraulic properties, and reported that the vadose zone could be divided into two parts (when the water table was situated sufficiently far from the soil surface) namely a highly transient zone near the soil surface, and a quasi-steady-state zone deeper in the soil. For low water tables, steady-state flow could hence reconstruct a "true" solute BTC at a control plane located near to the water table depth. This was reported to apply to heterogeneous soils, and in both the presence and absence of vegetation (Russo and Fiori 2008).

Homogeneous vs. heterogeneous flow

Non-equilibrium flow and transport in fractures, macro-pores or other types of high permeability zones are likely to have a large influence on microbial transport in the field (*e.g.*, Jamieson *et al.* (2002) and McMurry *et al.* (1998)). The sources of preferential flow could, for example, be tubular pores near the surface, due to soil fauna and flora roots as well as cracks due to clay shrinking and cracking (Carlander *et al.* 2000). Preferential flow is generally thought to reduce bacterial subsurface removal due to faster transport and reduced deposition in the soil matrix (Smith *et al.* 1985; de Jonge *et al.* 2004).

A variety of mathematical models and computer tools have been developed to describe flow and transport in structured soils. These have been reviewed by, *e.g.*, Simunek *et al.* (2003), Gerke (2006), Jarvis and Dubois (2006) and Köhne *et al.* (2009a; 2009b). The different models include descriptions of the porous medium as a single- or multiple continua or as a network (Köhne *et al.* 2009a; Köhne *et al.* 2009b). The group of multiple-continua models can be divided into the following: mobile–immobile models, in which the soil micropore network is considered to have such a low permeability that water is immobile in one direction; the dual-porosity model (Gerke and van Genuchten 1993) that assumes that the filter medium consists of two overlapping connected domains, in one the water flows rapidly (the macro-pore) and, in the other, transport is only due to diffusion (the matrix domain); and the dual-permeability model (Simunek *et al.* 2003), which adds the possibility of advection in the matrix (Coppola *et al.* 2009; Köhne *et al.* 2009a; Köhne *et al.* 2009b). For example, Jiang *et al.* (2010) used the dual-porosity formulation to describe water flow and bacterial transport. The equation applied is based on the assumption that Richard's equation applies to macro-pore flow, that a mass balance equation can describe the moisture dynamics in the immobile water region, and that the transfer rate for water exchange between the macro-pores and the matrix is driven by the difference in soil water pressure head (Gerke and van Genuchten 1993; Simunek *et al.* 2003). This approach demands that the pressure heads must be calculated for both the matrix and the macro-pores and that each region has its own values of θ_s, θ_r as well as *a* and *n*. The implication based on this is that eleven parameters must be calibrated to describe the transport: six for transport in the macro-pores, four for transport in the matrix, and one for the exchange between the two regions (Jiang *et al.* 2010). One type of network model is the discrete fracture model, which describes discrete flow in fractures and in fractured rock, in which water exchange between the fracture and the matrix is described as an advection-diffusion process (Köhne *et al.* 2009a). The resulting breakthrough curves display multiple peaks, due to channeling, *i.e.* concentration of fluid flow to preferential paths (Nordqvist *et al.* 1996). Discrete fracture models have, *e.g.*, been applied to assess safety in relation to geologic repositories for nuclear waste (*e.g.*, Min *et al.* (2005)). Typically, the discrete fractures and their individual characteristics are stochastically generated. The discrete approach is not practical for problems at the scale of kilometers, considering that that this would imply the calculation of an enormous amount of fractures (computer power is limited) (Min *et al.* 2005).

Nevertheless, as yet, knowledge with regards to the processes relating to the preferential flow and transport of microbes is incomplete, and there is no generally accepted, physical model for describing the related processes. Models are required to be locally calibrated. This complicates any comparative analyses and predictions with regards to new systems. Steady-state, homogeneous flow could, in some cases, provide realistic simplifications. Evaluating the flow and transport in partially saturated field soils, Cey *et al.* (2009), determined that worm burrows were the primary pathways and the major macro-pore type for the deep transport of dye and microspheres. For most plants, the limit of the root-zone is, approximately, 1 m below the ground surface (Pang 2009). The author defined vadose zones as the unsaturated subsurface media below soils and above the ground water table, devoid of animals, roots, or any other markers of biological activity and set its upper boundary at 1 m below the ground. Further, Cey *et al.* (2009) reported that the impact of macro-pore flow increased with the infiltration rate. In designed systems, management schemes could affect the impact of preferential flow paths (Jiang *et al.* 2010). Their influence could, for example, be significantly reduced in a filter system with a specific and relatively homogeneous filter material, without the presence of plant roots or worms, and a controlled, constant and low infiltration rate. On the subject of irrigation, Jiang *et al.* (2010) stated that the effect of preferential flow could be minimized through the adoption of appropriate schemes, for example including the use of spray instead of flood irrigation and a high irrigation frequency. In summation, these results indicate that the findings in *Paper II* and *Paper III* – applying to steady-state, homogeneous flow – could be relevant for natural conditions lower down in the soil, as well as in engineered systems with controlled flow rates and filter media.

Ignoring the impact of E. coli growth and the presence of biofilm (Paper II and Paper III)

The main reason why *E. coli* growth was ignored in *Paper II* and *Paper III* is that this was the case in the cited experimental studies that provided the basis for the simulations. In agreement to this, Foppen and Schijven (2006) state: "*when E. coli bacteria are introduced into water environments, they gradually die*". Moreover, assuming that soil is a long way from being saturated and that the water content is constant, recent studies indicate that the importance of bacterial growth in biofilm is limited. Habitats in unsaturated environments are fragmented and connected by means of thin water films. Bacterial colony expansion is driven by individual cell mobility, which is constrained by viscous and capillary forces; reduced water content decreases bacterial mobility due to pinning by capillary forces (Dechesne *et al.* 2010; Wang and Or 2010). Hence, biofilm formation and the related bacterial processes are likely to be more important in saturated systems, whereas physical filtration is still very important in filters that contain fine grains and significant amounts of air. Nevertheless, retention of *E. coli* in biofilms of other microbes can occur (Wang *et al.* 2011). In this case, removal mainly occurs in the biological layer that is formed in the top few centimeters of the sand filter (WHO 2011). However, accounting for such effects was not within the scope of *Paper II* and *Paper III*.

Additionally, Zhang *et al.* (2010) reported that colloids, once retained, acted as new deposition sites for other suspended colloids (clogging), and that the deposition rates were dependent on input concentration. Moreover, aggregations in the fluid, or flocs, could affect straining due to the larger size of the transported unit (Stevik *et al.* 1999a; Schinner *et al.* 2009). The impact of clogging and flocs could be important processes; however their importance can hardly be assessed in column or lysimeter studies unless the deposition profiles are analyzed, which is comparatively rare.

CONCLUSION

A deeper understanding of bacterial transport behavior in unsaturated filter media is imperative in order to provide for improved guidelines relating to groundwater protection and the construction of filter systems. The most important findings in this thesis, relating to the main removal processes, influencing factors and modeling approaches relevant for *E. coli* in unsaturated porous media are outlined below. Pore-scale imaging and integrated results indicate that the key removal mechanism in sand is straining at, or near, the solid-air-water interface, to which the bacteria are transported by means of hydrodynamic forces (in the presence of repulsive energy barriers, as is common in the natural environment). This mechanism could be preceded by weak, reversible attachment and *E. coli* swimming along a grain. It is, however, possible that the deposition behavior varies with the type of *E. coli* strain. As regards survival, it is probable that inactivation is the main process. Additionally, the impact of *E. coli* removal due to the presence of biofilms by other microbes is likely to be significant; however, this process is highly dependent on the surrounding conditions and it was beyond the scope of this paper to estimate its relative importance on removal.

Physical factors are likely to a have high influence on the spatial removal rate. These particularly affect straining processes near the solid-air water interface. More specifically, the results point to that the major influencing factors is the flow rate, which is dependent on the saturation, the filter media grain size and surface roughness. The results further indicate that the flow rate, as compared to the filter depth, has a larger influence on the attenuation efficiency. In addition, increased ionic strength could augment weak attachment and funneling to low-velocity regions. Inactivation increases with decreased moisture and nutrient content, as well as increased temperature.

It is not clear how to incorporate the findings on key processes and influencing factors in predictive models. In the literature, a multitude of models that account for bacterial attenuation have been proposed. The model structures and parameters used are often incoherent and alternative theories could describe the same observation. Moreover, the relevance of a model approach and the corresponding derived parameters in an earlier study, has rarely, if ever, been evaluated in a new one. It thus remains uncertain how literature models could be generalized and used for predictions in new scenarios, even under simple, controlled column scale conditions. A conservative approach includes the calculation of a wide range of outcomes and considers the worst case scenario. However, the ranges of the *E. coli* attenuation efficiencies in various sand filters were discovered to be extremely wide, when considering the breakthrough curves resulting from six different literature models (derived under similar experimental conditions). If the underlying processes are unknown, a pragmatic and reasonable approach could alternatively be a first-order kinetic model with constant temporal removal coefficients.

Suggestions for further research

Considering that the importance of the flow rate on removal has been underlined in this thesis, future research is recommended to include the effects of sudden transient flow, as well as structured filter media – even though bacterial fate under simplified conditions is not, as yet, sufficiently understood. Sudden imbibitions could, for example, cause both release of bacteria and colony expansion. Furthermore, for improved understanding of the underlying removal mechanisms, pore-scale imaging of the act of bacterial deposition is recommended, preferably in combination with a study of the related breakthrough curves and deposition profiles – ideally evaluated as dependent on time. In addition, in order to enable macro-scale predictions, theoretical up-scaling methodologies relating to unsaturated media require further development. The knowledge relating to *E. coli* biofilm development and its impact on attenuation in dynamic, unsaturated systems is also currently rather limited and requires further studies. Finally, clarification is required regarding *E. coli* as a relevant indicator bacterium when predicting the transport and removal of pathogenic bacteria in general.

REFERENCES

ARGOSS (2001). *Guidelines for Assessing the Risk to Groundwater from On-Site Sanitation. British Geological Survey Commissioned Report CR/01/142*. London, Natural Environment Research Council

Ausland, G., T. K. Stevik, J. F. Hanssen, J. C. Køhler and P. D. Jenssen (2002). *Intermittent filtration of wastewater--removal of fecal coliforms and fecal streptococci.* Water Research 36(14): 3507-3516.

Azadpour-Keeley, A. and C. H. Ward (2005). *Transport and survival of viruses in the subsurface - processes, experiments, and simulation models.* Remediation Journal 15(3): 23-49.

Bauman, W. J., A. Nocker, W. L. Jones and A. K. Camper (2009). *Retention of a model pathogen in a porous media biofilm.* Biofouling: The Journal of Bioadhesion and Biofilm Research 25(3): 229 - 240.

Baumann, T., L. Toops and R. Niessner (2010). *Colloid dispersion on the pore scale.* Water Research 44(4): 1246-1254.

Beven, K. and J. Freer (2001). *Equifinality, data assimilation, and uncertainty estimation in mechanistic modelling of complex environmental systems using the GLUE methodology.* Journal of Hydrology 249(1-4): 11-29.

Bhattacharjee, S., J. N. Ryan and M. Elimelech (2002). *Virus transport in physically and geochemically heterogeneous subsurface porous media.* Journal of Contaminant Hydrology 57(3-4): 161-187.

Bradford, S. A., M. Bettahar, J. Simunek and M. T. van Genuchten (2004). *Straining and Attachment of Colloids in Physically Heterogeneous Porous Media.* Vadose Zone Journal 3(2): 384-394.

Bradford, S. A., J. Simunek, M. Bettahar, M. T. van Genuchten and S. R. Yates (2003). *Modeling Colloid Attachment, Straining, and Exclusion in Saturated Porous Media.* Environmental Science & Technology 37(10): 2242-2250.

Bradford, S. A., J. Simunek, M. Bettahar, M. T. van Genuchten and S. R. Yates (2006). *Significance of straining in colloid deposition: Evidence and implications.* Water Resour. Res. 42(12): W12S15.

Bradford, S. A. and S. Torkzaban (2008). *Colloid Transport and Retention in Unsaturated Porous Media: A Review of Interface-, Collector-, and Pore-Scale Processes and Models.* Vadose Zone Journal 7(2): 667-681.

Branda, S. S., A. Vik, L. Friedman and R. Kolter (2005). *Biofilms: the matrix revisited.* Trends in Microbiology 13(1): 20-26.

Bridge, J. W., A. L. Heathwaite and S. A. Banwart (2009). *Measurement of Colloid Mobilization and Redeposition during Drainage in Quartz Sand.* Environmental Science & Technology 43(15): 5769-5775.

Brix, H. and C. A. Arias (2005). *The use of vertical flow constructed wetlands for on-site treatment of domestic wastewater: New Danish guidelines.* Ecological Engineering 25(5): 491-500.

Brooks, R. H. and A. T. Corey (1964). *Hydraulic properties of porous media*, Hydrology Paper No. 3, Civil Engineering Dep., Colorado State Univ., Fort Collins, CO, USA.

Burkhardt, M., R. Kasteel, J. Vanderborght and H. Vereecken (2008). *Field study on colloid transport using fluorescent microspheres.* European Journal of Soil Science 59(1): 82-93.

Carlander, A., P. Aronsson, G. Allestam, T. A. Stenström and K. Perttu (2000). *Transport and retention of bacteriophages in two types of willow-cropped lysimeters.* Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering 35(8): 1477 - 1492.

Carsel, R. F. and R. S. Parrish (1988). *Developing joint probability distributions of soil water retention characteristics.* Water Resour. Res. 24(5): 755-769.

Cey, E. E., D. L. Rudolph and J. Passmore (2009). *Influence of macroporosity on preferential solute and colloid transport in unsaturated field soils.* Journal of Contaminant Hydrology 107(1-2): 45-57.

Chabaud, S., Y. Andres, A. Lakel and P. Le Cloirec (2006). *Bacteria removal in septic effluent: Influence of biofilm and protozoa.* Water Research 40(16): 3109-3114.

Chen, G. (2008). *Bacterial interactions and transport in unsaturated porous media.* Colloids and Surfaces B: Biointerfaces 67(2): 265-271.

Chen, G., P. K. Subramaniam and K. Tawfiq (2010). *Bacterial deposition in unsaturated porous media as related to surface properties.* International Journal of Environment and Pollution 40(4): 363-379.

Chen, G. and H. Zhu (2005). *Bacterial adhesion to silica sand as related to Gibbs energy variations.* Colloids and Surfaces B: Biointerfaces 44(1): 41-48.

Cheng, T. and J. E. Saiers (2009). *Mobilization and transport of in situ colloids during drainage and imbibition of partially saturated sediments.* Water Resour. Res. 45(8): W08414.

COMSOL. (2008). *COMSOL Multiphysics Modeling Guide*. Version 3.5a.

Coppola, A., M. Kutílek and E. O. Frind (2009). *Transport in preferential flow domains of the soil porous system: Measurement, interpretation, modelling, and upscaling.* Journal of Contaminant Hydrology 104(1-4): 1-3.

Corapcioglu, M. Y. and H. Choi (1996). *Modeling Colloid Transport in Unsaturated Porous Media and Validation with Laboratory Column Data.* Water Resour. Res. 32(12): 3437-3449.

Crane, S. R. and J. A. Moore (1984). *Bacterial pollution of groundwater: A review.* Water, Air, & Soil Pollution 22(1): 67-83.

Crane, S. R. and J. A. Moore (1986). *Modeling enteric bacterial die-off: A review.* Water, Air, & Soil Pollution 27(3): 411-439.

Crist, J. T., J. F. McCarthy, Y. Zevi, P. Baveye, J. A. Throop and T. S. Steenhuis (2004). *Pore-Scale Visualization of Colloid Transport and Retention in Partly Saturated Porous Media.* Vadose Zone Journal 3(2): 444-450.

Crist, J. T., Y. Zevi, J. F. McCarthy, J. A. Throop and T. S. Steenhuis (2005). *Transport and Retention Mechanisms of Colloids in Partially Saturated Porous Media.* Vadose Zone Journal 4(1): 184-195.

de Jonge, L. W., C. Kjaergaard and P. Moldrup (2004). *Colloids and Colloid-Facilitated Transport of Contaminants in Soils: An Introduction.* Vadose Zone Journal 3(2): 321-325.

Dechesne, A., G. Wang, G. Gülez, D. Or and B. F. Smets (2010). *Hydration-controlled bacterial motility and dispersal on surfaces.* Proceedings of the National Academy of Sciences 107(32): 14369-14372.

DeNovio, N. M., J. E. Saiers and J. N. Ryan (2004). *Colloid Movement in Unsaturated Porous Media: Recent Advances and Future Directions.* Vadose Zone Journal 3(2): 338-351.

Derjaguin, B. V. and L. Landau (1941). *Theory of the stability of strongly charged lyophobic sols and of the adhesion of strongly charged particles in solution of electrolytes.* Acta Physicochim URSS 14: 633-662.

Emelko, M. B. and N. Tufenkji (2010). *Transport and fate of colloids and microbes in granular aqueous environments.* Water Research 44(4): 1027-1027.

Falvey, C. (1997). *Sand Filters Provide Quality, Low-Maintenance Treatment.* Pipeline. West Virginia University, Morgantown, WV, National Small Flows Clearinghouse. 8.

Foppen, J. W., G. Lutterodt, W. F. M. Röling and S. Uhlenbrook (2010). *Towards understanding inter-strain attachment variations of Escherichia coli during transport in saturated quartz sand.* Water Research 44(4): 1202-1212.

Foppen, J. W., M. van Herwerden and J. Schijven (2007). *Transport of Escherichia coli in saturated porous media: Dual mode deposition and intra-population heterogeneity.* Water Research 41(8): 1743-1753.

Foppen, J. W. A. and J. F. Schijven (2006). *Evaluation of data from the literature on the transport and survival of Escherichia coli and thermotolerant coliforms in aquifers under saturated conditions.* Water Research 40(3): 401-426.

Foppen, J. W. A., M. van Herwerden, M. Kebtie, A. Noman, J. F. Schijven, P. J. Stuyfzand and S. Uhlenbrook (2008). *Transport of Escherichia coli and solutes during waste water infiltration in an urban alluvial aquifer.* Journal of Contaminant Hydrology 95(1-2): 1-16.

Gao, B., J. E. Saiers and J. Ryan (2006). *Pore-scale mechanisms of colloid deposition and mobilization during steady and transient flow through unsaturated granular media.* Water Resour. Res. 42(1): W01410.

García, J., D. Rousseau, J. Morató, E. Lesage, V. Matamoros and J. Bayona (2010). *Contaminant Removal Processes in Subsurface-Flow Constructed Wetlands: A Review.* Critical Reviews in Environmental Science and Technology 40(7): 561 - 661.

Gargiulo, G., S. Bradford, J. Simunek, P. Ustohal, H. Vereecken and E. Klumpp (2007). *Bacteria transport and deposition under unsaturated conditions: The role of the matrix grain size and the bacteria surface protein.* Journal of Contaminant Hydrology 92(3-4): 255-273.

Gargiulo, G., S. A. Bradford, J. Simunek, P. Ustohal, H. Vereecken and E. Klumpp (2008). *Bacteria Transport and Deposition under Unsaturated Flow Conditions: The Role of Water Content and Bacteria Surface Hydrophobicity.* Vadose Zone Journal 7(2): 406-419.

Gerke, H. H. (2006). *Preferential flow descriptions for structured soils.* Journal of Plant Nutrition and Soil Science 169(3): 382-400.

Gerke, H. H. and M. T. van Genuchten (1993). *A Dual-Porosity Model for Simulating the Preferential Movement of Water and Solutes in Structured Porous Media.* Water Resour. Res. 29: 305-319.

Ginn, T. R., B. D. Wood, K. E. Nelson, T. D. Scheibe, E. M. Murphy and T. P. Clement (2002). *Processes in microbial transport in the natural subsurface.* Advances in Water Resources 25(8-12): 1017-1042.

Golfier, F., B. D. Wood, L. Orgogozo, M. Quintard and M. Buès (2009). *Biofilms in porous media: Development of macroscopic transport equations via volume averaging with closure for local mass equilibrium conditions.* Advances in Water Resources 32(3): 463-485.

Hall-Stoodley, L., J. W. Costerton and P. Stoodley (2004). *Bacterial biofilms: from the Natural environment to infectious diseases.* Nat Rev Micro 2(2): 95-108.

Hamilton, A. J., F. Stagnitti, X. Xiong, S. L. Kreidl, K. K. Benke and P. Maher (2007). *Wastewater Irrigation: The State of Play.* Vadose Zone Journal 6(4): 823-840.

Haznedaroglu, B. Z., H. N. Kim, S. A. Bradford and S. L. Walker (2009). *Relative Transport Behavior of Escherichia coli O157:H7 and Salmonella enterica Serovar Pullorum in Packed Bed Column Systems: Influence of Solution Chemistry and Cell Concentration.* Environmental Science & Technology 43(6): 1838-1844.

Howard, G., S. Pedley, M. Barrett, M. Nalubega and K. Johal (2003). *Risk factors contributing to microbiological contamination of shallow groundwater in Kampala, Uganda.* Water Research 37(14): 3421-3429.

Jamieson, R., R. Gordon, K. Sharples, G. Stratton and A. Madani (2002). *Movement and Persistence of Fecal Bacteria in Agricultural Soils and Subsurface Drainage Water: A review.* Canadian Biosystems Engineering 44: 6.1-6.10.

Jarvis, N. J. and I. G. Dubois. (2006). *State-of-the-art review of preferential flow. Report DL#6 of the FP6 EU-funded Functional Tools for Pesticide Risk Assessment and Management project.* Retrieved June 6th 2010, from http://www-eu-fottprint.org/downloads/FOOTPRINT_DL6.pdf.

Jiang, G., M. J. Noonan, G. D. Buchan and N. Smith (2007). *Transport of Escherichia coli through variably saturated sand columns and modeling approaches.* Journal of Contaminant Hydrology 93(1-4): 2-20.

Jiang, S., L. Pang, G. D. Buchan, J. Simunek, M. J. Noonan and M. E. Close (2010). *Modeling water flow and bacterial transport in undisturbed lysimeters under irrigations of dairy shed effluent and water using HYDRUS-1D.* Water Research 44(4): 1050-1061.

Jin, Y. and M. Flury (2002). *Fate and transport of viruses in porous media*. Advances in Agronomy, Academic Press(77): 39-102.

Johnson, W. P., E. Pazmino and H. Ma (2010). *Direct observations of colloid retention in granular media in the presence of energy barriers, and implications for inferred mechanisms from indirect observations.* Water Research 44(4): 1158-1169.

Keller, A. A. and M. Auset (2007). *A review of visualization techniques of biocolloid transport processes at the pore scale under saturated and unsaturated conditions.* Advances in Water Resources 30(6-7): 1392-1407.

Keller, A. A. and S. Sirivithayapakorn (2004). *Transport of colloids in unsaturated porous media: Explaining large-scale behavior based on pore-scale mechanisms.* Water Resour. Res. 40(12): W12403.

Kim, H. N., S. A. Bradford and S. L. Walker (2009). *Escherichia coli O157:H7 Transport in Saturated Porous Media: Role of Solution Chemistry and Surface Macromolecules.* Environmental Science & Technology 43(12): 4340-4347.

Kim, M.-K., S.-B. Kim and S.-J. Park (2008). *Bacteria transport in an unsaturated porous media: incorporation of air-water interface area model into transport modelling.* Hydrological Processes 22(13): 2370-2376.

King, E. L., K. Tuncay, P. Ortoleva and C. Meile (2009). *Modeling biogeochemical dynamics in porous media: Practical considerations of pore scale variability, reaction networks, and microbial population dynamics in a sandy aquifer.* Journal of Contaminant Hydrology In Press, Accepted Manuscript.

Ko, C.-H. and M. Elimelech (2000). *The "Shadow Effect" in Colloid Transport and Deposition Dynamics in Granular Porous Media: Measurements and Mechanisms.* Environmental Science & Technology 34(17): 3681-3689.

Kulabako, N. R., M. Nalubega and R. Thunvik (2007). *Study of the impact of land use and hydrogeological settings on the shallow groundwater quality in a peri-urban area of Kampala, Uganda.* Science of The Total Environment 381(1-3): 180-199.

Köhne, J. M., S. Köhne and J. Simunek (2009a). *A review of model applications for structured soils: a) Water flow and tracer transport.* Journal of Contaminant Hydrology 104(1-4): 4-35.

Köhne, J. M., S. Köhne and J. Simunek (2009b). *A review of model applications for structured soils: b) Pesticide transport.* Journal of Contaminant Hydrology 104(1-4): 36-60.

Lamb, R., K. Beven and S. Myrabø (1998). *Use of spatially distributed water table observations to constrain uncertainty in a rainfall-runoff model.* Advances in Water Resources 22(4): 305-317.

Langergraber, G. and J. Simunek (2005). *Modeling Variably Saturated Water Flow and Multicomponent Reactive Transport in Constructed Wetlands.* Vadose Zone Journal 4(4): 924-938.

Lazouskaya, V., Y. Jin and D. Or (2006). *Interfacial interactions and colloid retention under steady flows in a capillary channel.* Journal of Colloid and Interface Science 303(1): 171-184.

Lenhart, J. J. and J. E. Saiers (2002). *Transport of Silica Colloids through Unsaturated Porous Media: Experimental Results and Model Comparisons.* Environmental Science & Technology 36(4): 769-777.

Lewis, R. W., W. K. S. Pao and X.-S. Yang (2004). *Instability and reaction-diffusion transport of bacteria.* Communications in Numerical Methods in Engineering 20(10): 777-787.

Li, A. (2007). *Escherichia coli (E. coli) with Pili and Flagella (National University of Singapore).* Retrieved January 20th 2011, from http://www.icmm.csic.es/spmage/spmageview.php?id=50.

Li, Q., K. Ito, Z. Wu, C. S. Lowry and S. P. L. II (2009). *COMSOL Multiphysics: A Novel Approach to Ground Water Modeling.* Ground Water 47(4): 480-487.

Liu, Y. and J. Li (2008). *Role of Pseudomonas aeruginosa Biofilm in the Initial Adhesion, Growth and Detachment of Escherichia coli in Porous Media.* Environmental Science & Technology 42(2): 443-449.

Lutterodt, G., M. Basnet, J. W. A. Foppen and S. Uhlenbrook (2009). *The effect of surface characteristics on the transport of multiple Escherichia coli isolates in large scale columns of quartz sand.* Water Research 43(3): 595-604.

Macler, B. A. and J. C. Merkle (2000). *Current knowledge on groundwater microbial pathogens and their control.* Hydrogeology Journal 8(1): 29-40.

Matthess, G., A. Pekdeger and J. Schroeter (1988). *Persistence and transport of bacteria and viruses in groundwater -- a conceptual evaluation.* Journal of Contaminant Hydrology 2(2): 171-188.

McCarthy, J. F. and L. D. McKay (2004). *Colloid Transport in the Subsurface: Past, Present, and Future Challenges.* Vadose Zone Journal 3(2): 326-337.

McClaine, J. W. and R. M. Ford (2002a). *Characterizing the adhesion of motile and nonmotile Escherichia coli to a glass surface using a parallel-plate flow chamber.* Biotechnology and bioengineering 78(2): 179-189.

McClaine, J. W. and R. M. Ford (2002b). *Reversal of Flagellar Rotation Is Important in Initial Attachment of Escherichia coli to Glass in a Dynamic System with High- and Low-Ionic-Strength Buffers.* Appl. Environ. Microbiol. 68(3): 1280-1289.

McDowell-Boyer, L. M., J. R. Hunt and N. Sitar (1986). *Particle Transport Through Porous Media.* Water Resour. Res. 22(13): 1901-1921.

McMurry, S. W., M. S. Coyne and E. Perfect (1998). *Fecal Coliform Transport through Intact Soil Blocks Amended with Poultry Manure.* J Environ Qual 27(1): 86-92.

Min, K.-B., J. Rutqvist, C.-F. Tsang and L. Jing (2005). *Thermally induced mechanical and permeability changes around a nuclear waste repository—a far-field study based on equivalent properties determined by a discrete approach.* International Journal of Rock Mechanics and Mining Sciences 42(5-6): 765-780.

Molin, S. and V. Cvetkovic (2010). *Microbial risk assessment in heterogeneous aquifers: 1. Pathogen transport.* Water Resour. Res. 46(5): W05518.

Mosaddeghi, M. R., A. A. Mahboubi, S. Zandsalimi and A. Unc (2009). *Influence of organic waste type and soil structure on the bacterial filtration rates in unsaturated intact soil columns.* Journal of Environmental Management 90(2): 730-739.

Mosaddeghi, M. R., A. A. S. Sinegani, M. B. Farhangi, A. A. Mahboubi and A. Unc (2010). *Saturated and unsaturated transport of cow manure-borne Escherichia coli* *through in situ clay loam lysimeters*. Agriculture, Ecosystems & Environment 137(1-2): 163-171.

Murphy, E. M. and T. R. Ginn (2000). *Modeling microbial processes in porous media*. Hydrogeology Journal 8(1): 142-158.

Nasinyama, G. W., S. A. Mcewen, J. B. Wilson, D. Waltner-Toews, C. L. Gyles and J. Opuda-Asibo (2000). *Risk factors for acute diarrhoea among inhabitants of Kampala District, Uganda*. Rondebosch, AFRIQUE DU SUD, Health and Medical Publishing Group.

Nordqvist, A. W., Y. W. Tsang, C.-F. Tsang, B. Dverstorp and J. Andersson (1996). *Effects of high variance of fracture transmissivity on transport and sorption at different scales in a discrete model for fractured rocks*. Journal of Contaminant Hydrology 22(1-2): 39-66.

O'Connor, G. A., H. A. Elliott and R. K. Bastian (2008). *Degraded Water Reuse: An Overview*. J. Environ. Qual. 37(5_Supplement): S-157-S-168.

Ohshima, H. (1995). *Electrophoretic mobility of soft particles*. Colloids and Surfaces A: Physicochemical and Engineering Aspects 103(3): 249-255.

Ohshima, H. and T. Kondo (1991). *On the electrophoretic mobility of biological cells*. Biophysical Chemistry 39(2): 191-198.

Or, D., B. F. Smets, J. M. Wraith, A. Dechesne and S. P. Friedman (2007). *Physical constraints affecting bacterial habitats and activity in unsaturated porous media - a review*. Advances in Water Resources 30(6-7): 1505-1527.

Pang, L. (2009). *Microbial Removal Rates in Subsurface Media Estimated From Published Studies of Field Experiments and Large Intact Soil Cores*. J Environ Qual 38(4): 1531-1559.

Pang, L., M. Close, M. Goltz, L. Sinton, H. Davies, C. Hall and G. Stanton (2004). *Estimation of septic tank setback distances based on transport of E. coli and F-RNA phages*. Environment International 29(7): 907-921.

Pang, L., M. McLeod, J. Aislabie, J. Simunek, M. Close and R. Hector (2008). *Modeling Transport of Microbes in Ten Undisturbed Soils under Effluent Irrigation*. Vadose Zone Journal 7(1): 97-111.

Parkhurst, D. L. and C. A. J. Appelo (1999). *User's Guide to PHREEQC (version 2)—A Computer Program for Speciation, Batch-reaction, One-Dimensional Transport, and Inverse Geochemical Calculations, Water-Resources Investigations Report vol. 99–4259*. Denver, Colorado, U.S. Geological Survey, U.S. Department of the Interior.

Parkinson, J. S. (2009). *An overview of E. coli chemotaxis*. Retrieved January 17th 2011, from http://chemotaxis.biology.utah.edu/Parkinson_Lab/projects/ecolichemotaxis/ecolichemotaxis.html.

Passmore, J. M., D. L. Rudolph, M. M. F. Mesquita, E. E. Cey and M. B. Emelko (2010). *The utility of microspheres as surrogates for the transport of E. coli RS2g in partially saturated agricultural soil*. Water Research 44(4): 1235-1245.

Powelson, D. K. and A. L. Mills (2001). *Transport of Escherichia coli in Sand Columns with Constant and Changing Water Contents*. J Environ Qual 30(1): 238-245.

Pruess, K. (2004). *The TOUGH Codes--A Family of Simulation Tools for Multiphase Flow and Transport Processes in Permeable Media*. Vadose Zone Journal 3(3): 738-746.

Reddy, K. R., R. Khaleel and M. R. Overcash (1981). *Behavior and Transport of Microbial Pathogens and Indicator Organisms in Soils Treated with Organic Wastes*. J Environ Qual 10(3): 255-266.

Reichert, P. (1994). *AQUASIM—a tool for simulation and data analysis of aquatic systems*. Water Sci.
Technol. 30: 21–30.

Richards, L. A. (1931). *Capillary conduction of liquids through porous mediums.* Physics 1(5): 318-333.

Rockhold, M. L., R. R. Yarwood and J. S. Selker (2004). *Coupled Microbial and Transport Processes in Soils.* Vadose Zone Journal 3(2): 368-383.

Russo, D. and M. Bouton (1992). *Statistical analysis of spatial variability in unsaturated flow parameters.* Water Resour. Res. 28(7): 1911-1925.

Russo, D. and A. Fiori (2008). *Equivalent vadose zone steady state flow: An assessment of its capability to predict transport in a realistic combined vadose zone–groundwater flow system.* Water Resour. Res. 44(9): W09436.

Saiers, J. E. and J. J. Lenhart (2003). *Colloid mobilization and transport within unsaturated porous media under transient-flow conditions.* Water Resour. Res. 39(1): 1019.

Salvucci, A., W. Zhang, V. Morales, M. Cakmak, A. Hay and T. Steenhuis (2009). *The impact of biofilm-forming potential and tafi production on transport of environmental Salmonella through unsaturated porous media.* Biologia 64(3): 460-464.

Scandura, J. E. and M. D. Sobsey (1997). *Viral and bacterial contamination of groundwater from on-site sewage treatment systems.* Water Science and Technology 35(11-12): 141-146.

Schijven, J. F. and S. M. Hassanizadeh (2000). *Removal of Viruses by Soil Passage: Overview of Modeling, Processes, and Parameters.* Critical Reviews in Environmental Science and Technology 30(1): 49 - 127.

Schijven, J. F. and J. Simunek (2002). *Kinetic modeling of virus transport at the field scale.* Journal of Contaminant Hydrology 55(1-2): 113-135.

Schinner, T., A. Letzner, S. Liedtke, F. D. Castro, I. A. Eydelnant and N. Tufenkji (2009). *Transport of selected bacterial pathogens in agricultural soil and quartz sand.* Water Research 44(4): 1182-1192.

Schwartz, F. W. and H. Zhang (2003). *Fundamentals of Ground Water* John Wiley & Sons, 592 p.

Schäfer, A., P. Ustohal, H. Harms, F. Stauffer, T. Dracos and A. J. B. Zehnder (1998). *Transport of bacteria in unsaturated porous media.* Journal of Contaminant Hydrology 33(1-2): 149-169.

Sen, T. (2011). *Processes in Pathogenic Biocolloidal Contaminants Transport in Saturated and Unsaturated Porous Media: A Review.* Water, Air, & Soil Pollution 216(1): 239-256.

Shang, J., M. Flury, G. Chen and J. Zhuang (2008). *Impact of flow rate, water content, and capillary forces on in situ colloid mobilization during infiltration in unsaturated sediments.* Water Resour. Res. 44(6): W06411.

Shang, J., M. Flury and Y. Deng (2009). *Force measurements between particles and the air-water interface: Implications for particle mobilization in unsaturated porous media.* Water Resour. Res. 45(6): W06420.

Shein, E. and B. Devin (2007). *Current problems in the study of colloidal transport in soil.* Eurasian Soil Science 40(4): 399-408.

Simunek, J., C. He, L. Pang and S. A. Bradford (2006). *Colloid-Facilitated Solute Transport in Variably Saturated Porous Media: Numerical Model and Experimental Verification.* Vadose Zone Journal 5(3): 1035-1047.

Simunek, J., N. J. Jarvis, M. T. van Genuchten and A. Gärdenäs (2003). *Review and comparison of models for describing non-equilibrium and preferential flow and transport in the vadose zone.* Journal of Hydrology 272(1-4): 14-35.

Simunek, J., M. Sejna and M. T. Van Genuchten (1999a). *The HYDRUS-2D software package for simulating two-dimensional movement of water, heat and multiple solutes in variably-saturated porous media.* Golden, Colorado, International Gorund Water Modeling Center, Colorado School of Mines.

Simunek, J., M. T. van Genuchten and M. Sejna (2005). *The HYDRUS-1D software package for simulating the onedimensional movement of water, heat, and multiple solutes in variably-saturated media*. HYDRUS Software Series 3, Department of Environmental Sciences, University of California Riverside, Riverside, CA. 315 pp.

Simunek, J., M. T. van Genuchten, M. Sejna, N. Toride and F. J. Leij (1999b) *The STANMOD computer software for evaluating solute transport in porous media using analytical solutions of convection-dispersion equation. Versions 1.0 and 2.0. IGWMC - TPS - 71.*, 32 p.

Sirivithayapakorn, S. and A. Keller (2003). *Transport of colloids in unsaturated porous media: A pore-scale observation of processes during the dissolution of air-water interface.* Water Resour. Res. 39(12): 1346.

Sleytr, K., A. Tietz, G. Langergraber and R. Haberl (2007). *Investigation of bacterial removal during the filtration process in constructed wetlands.* Science of The Total Environment 380(1-3): 173-180.

Smith, J., B. Gao, H. Funabashi, T. N. Tran, D. Luo, B. A. Ahner, T. S. Steenhuis, A. G. Hay and M. T. Walter (2008). *Pore-Scale Quantification of Colloid Transport in Saturated Porous Media.* Environmental Science & Technology 42(2): 517-523.

Smith, M. S., G. W. Thomas, R. E. White and D. Ritonga (1985). *Transport of Escherichia coli Through Intact and Disturbed Soil Columns.* J Environ Qual 14(1): 87-91.

Steenhuis, T., A. Dathe, Y. Zevi, J. Smith, B. Gao, S. Shaw, D. DeAlwis, S. Amaro-Garcia, R. Fehrman, M. Ekrem Cakmak, I. Toevs, B. Liu, S. Beyer, J. Crist, A. Hay, B. Richards, D. DiCarlo and J. McCarthy (2006). *Biocolloid retention in partially saturated soils.* Biologia 61(0): S229-S233.

Stevik, T. K., G. Ausland, J. F. Hanssen and P. D. Jenssen (1999a). *The influence of physical and chemical factors on the transport of E. coli through biological filters for wastewater purification.* Water Research 33(18): 3701-3706.

Stevik, T. K., G. Ausland, P. D. Jenssen and R. L. Siegrist (1999b). *Removal of E. coli during intermittent filtration of wastewater effluent as affected by dosing rate and media type.* Water Research 33(9): 2088-2098.

Stevik, T. K., A. Kari, G. Ausland and J. Fredrik Hanssen (2004). *Retention and removal of pathogenic bacteria in wastewater percolating through porous media: a review.* Water Research 38(6): 1355-1367.

Strathmann, M., C. F. Leon-Morales and H.-C. Flemming (2007). *Influence of Biofilms on Colloid Mobility in the Subsurface.* Colloidal Transport in Porous Media. F. H. Frimmel, F. Kammer and H.-C. Flemming, Springer Berlin Heidelberg: 143-173.

Sun, N., M. Elimelech, N.-Z. Sun and J. N. Ryan (2001). *A novel two-dimensional model for colloid transport in physically and geochemically heterogeneous porous media.* Journal of Contaminant Hydrology 49(3-4): 173-199.

Taylor, R., A. Cronin, S. Pedley, J. Barker and T. Atkinson (2004). *The implications of groundwater velocity variations on microbial transport and wellhead protection - review of field evidence.* FEMS Microbiology Ecology 49(1): 17-26.

Toride, N., M. Inoue and F. J. Leij (2003). *Hydrodynamic Dispersion in an Unsaturated Dune Sand.* Soil Sci Soc Am J 67(3): 703-712.

Toride, N., F. J. Leij and M. T. van Genuchten (1995). *The CXTFIT code for estimating transport parameters from laboratory or field tracer experiments. Version 2.0. Research Report No. 137.* Riverside, California, U.S. Salinity Laboratory, USDA, ARS.: 121 pp.

Torkzaban, S., S. A. Bradford, M. T. van Genuchten and S. L. Walker (2008a). *Colloid transport in unsaturated porous media: The role of water content and ionic strength on particle straining.* Journal of Contaminant Hydrology 96(1-4): 113-127.

Torkzaban, S., S. S. Tazehkand, S. L. Walker and S. A. Bradford (2008b). *Transport and fate of bacteria in porous media: Coupled effects of chemical conditions and pore space geometry.* Water Resour. Res. 44(4): W04403.

Trefry, M. G. and I.-u. Haque (2010). *Clean Groundwater Underpins the Developing World.* Ground Water 48(2): 170-170.

Tsang, C.-F., L. Gelhar, G. de Marsily and J. Andersson (1994). *Solute transport in heterogeneous media: A discussion of technical issues coupling site characterization and predictive assessment.* Advances in Water Resources 17(4): 259-264.

Tufenkji, N. (2007). *Modeling microbial transport in porous media: Traditional approaches and recent developments.* Advances in Water Resources 30(6-7): 1455-1469.

Tufenkji, N. and M. Elimelech (2004). *Deviation from the Classical Colloid Filtration Theory in the Presence of Repulsive DLVO Interactions.* Langmuir 20(25): 10818-10828.

Unc, A. and M. J. Goss (2003). *Movement of Faecal Bacteria through the Vadose Zone.* Water, Air, & Soil Pollution 149(1): 327-337.

USEPA (2003). *Bacterial water quality standards for recreational waters (fresh and marine). Status report. EPA/823/R-03/008. Office of Water, U.S. Environmental Protection Agency, Washington, D.C.*

Vacca, G., H. Wand, M. Nikolausz, P. Kuschk and M. Kästner (2005). *Effect of plants and filter materials on bacteria removal in pilot-scale constructed wetlands.* Water Research 39(7): 1361-1373.

van Genuchten, M. T. (1980). *A closed-form equation for predicting the hydraulic conductivity of unsaturated soils.* Soil Science Society of America Journal 44: 892-898.

Wan, J. and T. K. Tokunaga (1997). *Film Straining of Colloids in Unsaturated Porous Media: Conceptual Model and Experimental Testing.* Environmental Science & Technology 31(8): 2413-2420.

Wan, J. and J. L. Wilson (1994a). *Colloid transport in unsaturated porous media.* Water Resour. Res. 30(4): 857-864.

Wan, J. and J. L. Wilson (1994b). *Visualization of the role of the gas-water interface on the fate and transport of colloids in porous media.* Water Resour. Res. 30(1): 11-23.

Wan, J., J. L. Wilson and T. L. Kieft (1994). *Influence of the Gas-Water Interface on Transport of Microorganisms through Unsaturated Porous Media.* Appl. Environ. Microbiol. 60(2): 509-516.

Wand, H., G. Vacca, P. Kuschk, M. Krüger and M. Kästner (2007). *Removal of bacteria by filtration in planted and non-planted sand columns.* Water Research 41(1): 159-167.

Wang, A., B. Lin, B. E. Sleep and S. N. Liss (2011). *The Impact of Biofilm Growth on Transport of Escherichia coli O157:H7 in Sand.* Ground Water 49(1): 20-31.

Wang, G. and D. Or (2010). *Aqueous films limit bacterial cell motility and colony expansion on partially saturated rough surfaces.* Environmental Microbiology 12(5): 1363-1373.

Wang, M. and R. M. Ford (2009). *Transverse Bacterial Migration Induced by Chemotaxis in a Packed Column with Structured Physical Heterogeneity.* Environmental Science & Technology 43(15): 5921-5927.

Wang, W. and M. Zhou (2010). *Degradation of trichloroethylene using solvent-responsive polymer coated Fe nanoparticles.* Colloids and Surfaces A: Physicochemical and Engineering Aspects 369(1-3): 232-239.

Vega, E., B. Lesikar and S. D. Pillai (2003). *Transport and survival of bacterial and viral tracers through submerged-flow constructed wetland and sand-filter system.* Bioresource Technology 89(1): 49-56.

Verwey, E. J. W. and J. T. G. Overbeek (1948). *Theory of the Stability of Lyophobic Colloids*, Amsterdam: Elsevier.

WHO (2003). *World Health Report*. W. H. Organization.

WHO (2008). *World Health Organization Guidelines for Drinking-water Quality 3rd Edition*. Geneva, WHO Press.

WHO. (2009). *Diarrhoeal Diseases.* Retrieved January 25th 2010, 2010, from http://www.who.int/vaccine_research/diseases/diarrhoeal/en/index.html.

WHO (2011). *World Health Organization Guidelines for Drinking-water Quality 4th Edition*. Geneva, WHO Press.

Vigeant, M. A.-S., R. M. Ford, M. Wagner and L. K. Tamm (2002). *Reversible and Irreversible Adhesion of Motile Escherichia coli Cells Analyzed by Total Internal Reflection Aqueous Fluorescence Microscopy.* Appl. Environ. Microbiol. 68(6): 2794-2801.

Winston, R. B. (2010). *Online Guide to MODFLOW* Retrieved May 23rd, 2010, from http://water.usgs.gov/nrp/gwsoftware/modflow2000/MFDOC/index.html.

Wood, B. D., M. Quintard and S. Whitaker (2002). *Calculation of effective diffusivities for biofilms and tissues.* Biotechnology and bioengineering 77(5): 495-516.

Wood, B. D. and S. Whitaker (1999). *Cellular growth in biofilms.* Biotechnology and bioengineering 64(6): 656-670.

Wood, B. D. and S. Whitaker (2000). *Multi-species diffusion and reaction in biofilms and cellular media.* Chemical Engineering Science 55(17): 3397-3418.

Yang, G. C. C., H.-C. Tu and C.-H. Hung (2007). *Stability of nanoiron slurries and their transport in the subsurface environment.* Separation and Purification Technology 58(1): 166-172.

Yang, H.-H., J. B. Morrow, D. Grasso, R. T. Vinopal and B. F. Smets (2006). *Intestinal versus External Growth Conditions Change the Surficial Properties in a Collection of Environmental Escherichia coli Isolates.* Environmental Science & Technology 40(22): 6976-6982.

Yao, K.-M., M. T. Habibian and C. R. O'Melia (1971). *Water and waste water filtration. Concepts and applications.* Environmental Science & Technology 5(11): 1105-1112.

Yates, M. V., S. R. Yates and C. P. Gerba (1988). *Modeling microbial fate in the subsurface environment.* Critical Reviews in Environmental Control 17(4): 307 - 344.

Yavuz Corapcioglu, M. and A. Haridas (1985). *Microbial transport in soils and groundwater: A numerical model.* Advances in Water Resources 8(4): 188-200.

Zevi, Y., A. Dathe, B. Gao, B. K. Richards and T. S. Steenhuis (2006). *Quantifying colloid retention in partially saturated porous media.* Water Resour. Res. 42(12): W12S03.

Zevi, Y., A. Dathe, B. Gao, W. Zhang, B. K. Richards and T. S. Steenhuis (2009). *Transport and retention of colloidal particles in partially saturated porous media: Effect of ionic strength.* Water Resour. Res. 45(12): W12403.

Zevi, Y., A. Dathe, J. F. McCarthy, B. K. Richards and T. S. Steenhuis (2005). *Distribution of Colloid Particles onto Interfaces in Partially Saturated Sand.* Environmental Science & Technology 39(18): 7055-7064.

Zhang, W., V. n. L. Morales, M. E. Cakmak, A. E. Salvucci, L. D. Geohring, A. G. Hay, J.-Y. Parlange and T. S. Steenhuis (2010). *Colloid Transport and Retention in Unsaturated Porous Media: Effect of Colloid Input Concentration.* Environmental Science & Technology 44(13): 4965-4972.

Zheng, C. and P. P. Wang (1999) *MT3DMS, A modular three-dimensional multi-species transport model for simulation of advection, dispersion and chemical reactions of contaminants in groundwater systems; documentation and user's guide. U.S. Army Engineer Research and Development Center Contract Report SERDP-99-1*. 202 p.

Zhuang, J., N. Goeppert, C. Tu, J. McCarthy, E. Perfect and L. McKay (2010). *Colloid transport with wetting fronts: Interactive effects of solution surface tension and ionic strength*. Water Research 44(4): 1270-1278.

Zhuang, J., J. S. Tyner and E. Perfect (2009). *Colloid transport and remobilization in porous media during infiltration and drainage*. Journal of Hydrology 377(1-2): 112-119.

Åkesson, A. (2010). *Hydraulic- hydromorphologic analysis as an aid for improving peak flow predictions, TRITA-LWR LIC 2051*. Land and Water Resources Engineering. Stockholm, Royal Institute of Technology.