

Transport and Fate of Microbial Pathogens in Agricultural Settings

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An understanding of the transport and survival of microbial pathogens (pathogens hereafter) in agricultural settings is needed to assess the risk of pathogen contamination to water and food resources, and to develop control strategies and treatment options. However, many knowledge gaps still remain in predicting the fate and transport of pathogens in runoff water, and then through the shallow vadose zone and groundwater. A number of transport pathways, processes, factors, and mathematical models often are needed to describe pathogen fate in agricultural settings. The level of complexity is dramatically enhanced by soil heterogeneity, as well as by temporal variability in temperature, water inputs, and pathogen sources. There is substantial variability in pathogen migration pathways, leading to changes in the dominant processes that control pathogen transport over different spatial and temporal scales. For

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example, intense rainfall events can generate runoff and preferential flow that can rapidly transport pathogens. Pathogens that survive for extended periods of time have a greatly enhanced probability of remaining viable when subjected to such rapid-transport events. Conversely, in dry seasons, pathogen transport depends more strongly on retention at diverse environmental surfaces controlled by a multitude of coupled physical, chemical, and microbiological factors. These interactions are incompletely characterized, leading to a lack of consensus on the proper mathematical framework to model pathogen transport even at the column scale. In addition, little is known about how to quantify transport and survival parameters at the scale of agricultural fields or watersheds. This review summarizes current conceptual and quantitative models for pathogen transport and fate in agricultural settings over a wide range of spatial and temporal scales. The authors also discuss the benefits that can be realized by improved modeling, and potential treatments to mitigate the risk of waterborne disease transmission.

KEY WORDS: models, pathogen, retention, survival, transport

1. INTRODUCTION

The health risks that pathogens pose to water and food resources are highly dependent on their transport and survival in agricultural settings. An understanding of the factors that influence pathogen fate in agricultural settings is therefore needed to assess the risk of pathogen contamination, and to develop control strategies to minimize pathogen migration. A significant amount of work has been done to elucidate the physical, chemical, and biological factors that control the transport and survival of pathogenic microorganisms (see reviews by Bradford and Torkzaban, 2008; de Jonge et al., 2004; DeNovio et al., 2004; Ferguson et al., 2003; Foppen and Schijven, 2006; Ginn et al., 2002; Harvey and Harms, 2002; Herzig et al., 1970; Jamieson et al., 2002; Jin and Flury, 2002; Khilar and Fogler, 1998; McCarthy and Zachara, 1989; McDowell-Boyer et al., 1986; Oliver et al., 2005; Pachepsky et al., 2006; Pang, 2009; Rockhold et al., 2004; Ryan and Elimelech, 1996; Schijven and Hassanizadeh, 2000; Sen and Khilar, 2006; Tufenkji et al., 2006; Tyrrel and Quinton, 2003; 2004; Unc and Goss, 2004). The vast majority of prior research has been conducted in small-scale laboratory experiments.

Despite extensive prior research, many gaps still remain in our knowledge of pathogen fate and transport in runoff water, through the shallow vadose zone, and groundwater. Generally speaking, our ability to predict pathogen transmission over long distances in watersheds remains poor. This is due to the large number of transport pathways through which pathogens can migrate, and the diverse array of processes that influence pathogen fate in agricultural settings. Relevant environmental scales range from soil pores

to agricultural fields, groundwater aquifers, and entire watersheds. In addition, the dominant transport and fate processes change with scale, as these depend on interactions with diverse environmental surfaces and transport pathways, both of which are poorly characterized. Little is also known about how to quantify pathogen transport and survival at field and watershed scales. Because available transport models inadequately describe microbial transport in both engineered systems and complex natural environments (e.g., Bradford and Torkzaban, 2008; Ginn et al., 2002), a wide range of biological and abiotic processes are being studied in increasing detail with the aim of finding universally applicable mathematical expressions that will account for the unresolved discrepancies.

This review summarizes the state of the science with regard to pathogen transport and fate in agricultural settings. The text is organized into four main sections. Section 1 presents a brief overview of pathogen sources and disease outbreaks in agricultural settings. Section 2 reviews the current conceptual understanding of pathogen transport and fate at various scales. Section 3 discusses available mathematical models for pathogen migration and survival. Section 4 considers commonly used treatments to minimize the risk of pathogen dissemination in agricultural systems. This review is not intended to be exhaustive, but rather outlines current approaches, potential benefits, and challenges of better understanding transport and fate of microbial pathogens in agricultural settings.

2. PATHOGENS

In the widest sense, a pathogen is an infectious microorganism that invades and affects disease to its host. This review is restricted to zoonotic microbial pathogens—organisms capable of causing disease in both animals and humans—commonly associated with agriculture. These organisms primarily comprise viruses, bacteria, and protozoal cysts. In this section, we review agricultural pathogen sources and related disease outbreaks.

2.1 Pathogens Sources

Fecal waste from humans, domesticated animals, wildlife, and insects frequently contain high concentrations of pathogenic microorganisms (Gerba and Smith, 2005; U.S. Department of Agriculture [USDA], 1992; U.S. Environmental Protection Agency [USEPA], 1998). In agricultural settings, improperly treated or poorly contained waste is often the primary source for pathogens. The pathogen concentration in animal waste is reported to depend on the species, age, health, stress, and diet (Nicholson et al., 2000). In addition, only a small fraction of the pathogen host population may be infected with a specific pathogen at any given time, and this fraction will change over the course of a year. The U.S. Environmental Protection Agency (USEPA) has attributed much of the pathogen contamination of water bodies to manure production

at concentrated animal feeding operations (CAFOs; USEPA, 2004). Each of these farms produces large amounts of manure and wastewater. According to the U.S. Department of Agriculture (USDA), the amount of manure generated at CAFOs is estimated to exceed 335 million tons of dry matter per year (Kellogg et al., 2000; USDA, 2010). In comparison, the 307 million citizens of the United States produce around 18.1 million tons of dry matter per year that undergoes extensive treatment to kill pathogens before disposal and/or land application. More dispersed sources of pathogens in agricultural settings include wildlife, insects, and domesticated animals grazing rangelands and pastures. The most prevalent pathogen sources will therefore vary with land use (e.g., agricultural vs. urban), agricultural practices, and indigenous wildlife within a watershed.

Pathogen contamination in agricultural settings can originate from point or nonpoint sources. Point sources of fecal contamination include leaking septic and sewer systems, drains, manure piles, or wastewater lagoons. In contrast, nonpoint sources are more difficult to characterize because they involve spatial and/or temporal variability in pathogen sources, as well as spatial translocation in the environment external to a host. General releases of fecal contamination in agricultural settings appear to be largely from nonpoint sources (Bowman, 2010). It is therefore extremely difficult to determine whether the pathogen source in many outbreaks originates from farm animals, wildlife, or humans. However, surveys of waterborne disease outbreaks frequently demonstrate a farm animal source (Centers for Disease Control and Prevention, 1998). The USEPA ranks agriculture as the most probable source of threatened or impaired rivers and streams (USEPA, 2010).

Manure and wastewater from CAFOs are routinely applied to agricultural fields as a soil amendment based on approved Nutrient Management Plans (NMPs) with little or no treatment to inactivate pathogens (Bradford et al., 2008; Bradford and Segal, 2009). If these wastes are inadequately treated before land application, then viable pathogens may survive or grow in field soils and contaminate plants (Berg et al., 2005; Reddy et al., 1981). Manure particles and pathogens may become suspended into water following precipitation events or via interception with flowing water (Bradford and Schijven, 2002). Surface water supplies may subsequently become contaminated as a result of runoff from agricultural lands and manure storage areas, interactions between surface water and water in the subsurface, and/or resuspension of contaminated soil and/or stream sediments. Groundwater may become contaminated as a result of recharge, groundwater/surface water interactions (such as river bank filtration), and groundwater pumping and capture from a pathogen source (i.e., recharge or surface water).

2.2 Disease Outbreaks

Surface and groundwater contamination by pathogens is common in many areas of the United States (Abbaszadegan et al., 2003; Borchardt et al.,

2003; USEPA, 1997). Drinking water exposure to pathogens may occur in vulnerable private wells (Macler and Merkle, 2000). Recreational exposure and illness can also occur in contaminated surface waters due to accidental ingestion or dermal contact (Yoder et al., 2008). Risk assessments have been performed to estimate the magnitude of endemic illness from pathogen contamination of groundwater by considering available information on exposure, contamination, and dose response. Pathogenic microorganisms in groundwater have been estimated to cause between 750,000 and 5 million illnesses in the United States per year (Macler and Merkle, 2000). Greater risks of serious illness occur for the very young, elderly, pregnant women, the immunocompromised, and those predisposed with other illnesses (Gerba, 1996).

A variety of recent studies have also identified several nonobvious waterborne transmission pathways via irrigation and food processing (Cotruvo et al., 2004; Doyle, 1990; Hanning et al., 2009; Lightfoot, 2004). For example, pathogens in manure and contaminated water resources have been implicated in foodborne disease outbreaks on a variety of fresh produce (Gerba and Smith, 2005; Lynch et al., 2009). Water used to irrigate crops comes from a wide variety of surface water and groundwater sources of different microbial quality (Gerba and Choi, 2006) that may foul field soils and fresh produce (Solomon et al., 2002; Solomon et al., 2003). The safety of fresh produce may be compromised by contact with contaminated soil or water (USFDA, 1998) as pathogens attach to and colonize the plant tissue (Kim and Harrison, 2008; Klerks et al., 2007; Ugoji et al., 2005). Another pathway for water-to-food-borne transmission of bacterial pathogens stems from terrestrial pathogen loading into surface waters, as has been identified for shellfish contaminated by fecal pathogens from river outflow (Cotruvo et al., 2004; Hackney and Potter, 1994a, 1994b; Till and McBride, 2004).

The World Health Organization (WHO) estimated that diarrheal diseases account for 4.1% of the total daily global disease burden and is responsible for the deaths of 1.8 million people every year (World Health Organization [WHO], 2004). Foodborne diseases in the United States have been estimated to cause approximately 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths per year (Mead et al., 1999). The contribution of pathogen contaminated water resources to foodborne diseases is unknown. However, Lynch et al. (2006) reported that vegetables, fruits, and nuts accounted for 4.0–4.7% of the foodborne disease outbreaks in the United States between 1999 and 2002, yet 31.1 to 41.3% of the total foodborne outbreaks have undetermined causes.

Food- and waterborne disease outbreaks have been associated with a variety of pathogenic bacteria, viruses, and protozoa (Yoder et al., 2008). The most significant manure-borne zoonotic pathogens include the Shiga toxin producing *Escherichia coli* strains (STEC) such as O157:H7, *Salmonella* spp.,

Campylobacter spp., *Listeria monocytogenes*, and the protozoan parasites *Cryptosporidium parvum* and *Giardia duodenalis* (Bowman and Bowman, 2009; Tyrrel and Quinton, 2003). Protozoal cyst-forming parasites became a major concern following recognition of their prevalence and persistence in the environment, realization that these pathogens were not effectively inactivated by conventional water treatment methods, and occurrence of several major waterborne disease outbreaks (Eisenberg et al., 2005; LeChevallier et al., 1991a, 1991b; Mac Kenzie et al., 1994; Robertson et al., 1992). STEC has recently received considerable attention because of numerous well-publicized outbreaks (Mølbak and Scheutz, 2004; Pennington, 2010; Tarr et al., 2005). *Campylobacter* and *Salmonella* are thought to be the most common causes of bacterial gastroenteritis worldwide, each responsible for the infection of several million cases in the United States each year (Cotruvo et al., 2004; Mead et al., 1999; USFDA, 1992). A variety of viruses are also potentially zoonotic. Noroviruses are the predominant cause of foodborne gastroenteritis worldwide. This type of virus has a wide range of animal hosts, with reported incidents in humans, pigs, cattle, and mice (Mattison et al., 2007). However, the contribution to human illness by norovirus remains unclear. More recently, increasing evidence has been gathered indicating that hepatitis E virus is zoonotic (Meng et al., 2002).

Quantitative determination of pathogen concentrations in complex environmental samples (manure, soil, and sediment) is extremely difficult and costly because it requires specialized equipment and personnel. Typical methods used to determine pathogen concentrations rely on fluorescent stains (e.g., DAPI [4',6-diamidino-2-phenylindole]/propidium iodide [PI] vital dyes, fluorescence in situ hybridization [FISH]), monitoring of specific activity (e.g., in vitro excystation analyses, coliform forming unit capabilities, respiratory activity), or extraction and amplification of nucleic acids (e.g., reverse transcription-polymerase chain reaction). These techniques frequently only produce semiquantitative pathogen concentrations because of method-specific limitations. For example, the extraction efficiency and representativeness of sampled organisms significantly affect the stain-type assay validity, but are difficult to evaluate. Methods that monitor specific activity require metabolically active and/or cultivable organisms, so the interpretation of these assays is clearly skewed. Keer and Birch (2003) noted that the number of viable organisms may be severely underrepresented by sublethally damaged cells, uncultivable strains, and viable but not culturable organisms that will go undetected by most viability assays. Alternatively, the use of DNA and RNA sequences can overestimate viability measurements, as nucleic acids from actively killed cells has been shown to persist for significant periods of time (Masters et al., 1994). Because of the aforementioned difficulties in determining pathogen concentrations in environmental samples, regulations to protect public health from pathogens are largely based on assays catered to specific indicator microorganisms such as total or fecal coliform (FC), *Enterococcus*, and *E. coli*.

3. CONCEPTUAL OVERVIEW OF PATHOGEN TRANSPORT AND FATE

3.1 Pathogen Interactions With Environmental Surfaces

Interactions between pathogens and environmental surfaces play a critical role in many environmental transport and fate processes such as retention, release, aggregation, and survival. Interaction energies depend on the properties of the pathogens, interfaces, and the aqueous phase. Attractive interaction energy of pathogens with the solid-water interface (SWI), the air-water interface (AWI), and the stability of colloid suspensions has frequently been quantified using theory developed by Derjaguin-Landau-Verwey-Overbeek (DLVO; Derjaguin and Landau, 1941; Verwey and Overbeek, 1948). DLVO theory considers that the interaction energy between surfaces is controlled by the sum of short-range van der Waals and electrostatic interactions. Provided below is a brief discussion of DLVO theory. Detailed information and discussion about the theory has been extensively covered elsewhere (Elimelech et al., 1995; Israelachvili, 1992).

Calculated DLVO interaction energy profiles are used to determine if conditions are unfavorable (presence of high energy barriers) or favorable (absence of energy barriers and/or presence of energy minima) for attachment. Unfavorable interaction conditions occur in many natural environments because most pathogens and solid surfaces are negatively charged at prevailing pH conditions (Wan and Tokunaga, 2002). However, pathogens may still be attracted to an interface at short separation distances when attractive van der Waals interactions exceed repulsive electrostatic forces. Short-range interactions can result in weak attraction at secondary minima wells, or strong attraction at primary minima wells (Franchi and O'Melia, 2003; Hahn et al., 2004; Kuznar and Elimelech, 2007; Tufenkji and Elimelech, 2005a).

Transitions between favorable and unfavorable conditions are most commonly induced by changes in solution chemistry. A decrease in the ionic strength (IS) will result in an expansion of the double layer thickness and an increase in the magnitude of the surface potential (Elimelech et al., 1995; Israelachvili, 1992). Increases in the solution pH will increase the electrostatic repulsion; e.g., changes in surface potentials can result from deprotonation of pH-dependent charge sites (Bohn et al., 1985; Ryan and Elimelech, 1996). Ion exchange will influence the solution IS, double layer thickness, and surface potential due to their dependence on the ion valence. Divalent ions in solution will increase the solution IS and decrease the double layer thickness to a greater degree compared with similar concentrations of monovalent ions (Elimelech et al., 1995; Israelachvili, 1992). In addition to screening the charge with counter ions, certain nonindifferent electrolytes (e.g., divalent ions) adsorb onto charged surfaces to neutralize the charge and consequently reduce the magnitude of the surface potential (Elimelech

et al., 1995; Israelachvili, 1992; Khilar and Fogler, 1998). For the previously listed reasons, an increase in the attachment efficiency of pathogens is expected at lower pH (when functional groups are protonated), at higher IS (when the charge is screened), and in the presence of higher relative concentrations of divalent ions (when the charge is neutralized) (Elimelech et al., 1995; Israelachvili, 1992).

DLVO calculations employ information from measured or estimated zeta potential of the microbial suspension and the porous medium. The zeta potential is a macroscopic, empirical (measurement-based) parameter used to represent the charge of hard and impermeable particles. Zeta potentials at best reflect only the net electrokinetic properties of microbes and the solid-water interface (SWI; Foppen and Schijven, 2006). The charge of the SWI mainly depends on the properties of the soils, including soil mineralogy, adsorbed organic matter, exchangeable ions, metal oxides and clays. Intrinsic surface impurities of the porous medium can generate localized regions where attractive interaction is favorable even when the bulk surface has unfavorable properties (Kim et al., 2008; Metge et al., 2010). Thus, soil surfaces coated with clay particles or iron or aluminum oxides offer more retention sites for negatively charged microorganisms (Abudalo et al., 2005; Attinti et al., 2010; Kim et al., 2008; Syngouna and Chrysikopoulos, 2010). Nanoscale heterogeneities have often been implicated in attachment under such conditions (Bhattacharjee et al., 1998; Duffadar and Davis, 2007, 2008; Duffadar et al., 2009; Hoek and Agarwal, 2006; Kozlova and Santore, 2006; Kalasin and Santore, 2008; Santore and Kozlova, 2007; Suresh and Walz, 1996). The grid surface integration technique has been employed to explicitly account for variations in surface charge, shape, and roughness on the interaction energy (Duffadar and Davis, 2007; Hoek and Agarwal, 2006).

Classic DLVO theory assumes inert spherical colloids with surfaces that are rigid, smooth, and uniformly charged. However, microbes have uneven surface charge, hydrophobic properties, extracellular polymeric substances (EPS; e.g., proteins, lipopolysaccharides, extracellular polysaccharides), extracellular structures (e.g., pili and flagella), and irregular cell shape (Camesano and Logan, 2000; Ginn et al., 2002; Kim et al., 2009a; Kim et al., 2009b). Therefore, microbes generally show more complex surface interactions relative to inorganic particles. Moreover, microbial cells dynamically adjust to changes in the surrounding microenvironment because they are soft and permeable. The surface charge of microorganisms may be dynamic and unevenly distributed, and microbial surface properties are known to vary according to the strain and growth phase of the cell (Foppen and Schijven, 2006). Hence, many assumptions of DLVO theory do not apply to microorganisms. Considering all of these complexities is challenging, and additional research is warranted to better quantify and predict pathogen interactions with heterogeneous environmental surfaces.

Adhesive interactions may be enhanced or diminished by interactions that are not considered in traditional DLVO theory, such as Born, hydrophobic, hydration, steric, Lewis acid-base interactions, and capillary forces (Bradford and Torkzaban, 2008; Elimelech et al., 1995; Israelachvili, 1992; Grasso et al., 2002; Norde and Lyklema, 1989; Ryan and Elimelech, 1996; van Oss, 2003; van Oss et al., 1986; van Oss et al., 1987). Energy calculations including these additional interactions are commonly referred to as extended DLVO (XDLVO) energies. Subsequently, we discuss several of these XDLVO interactions that are most relevant to pathogens. Additional information on XDLVO interactions is provided by Israelachvili (1992) and Elimelech et al. (1995).

Many microorganisms may be subject to steric interactions because of their surface macromolecules and structures. Steric interactions result from the sorption of chains and/or chain elements onto surfaces (Rijnaarts et al., 1999). The magnitude of steric forces depends on the inter-chain strand changes in osmotic pressure, chain length, charge, and elasticity (Bowen and Williams, 1995; Ohshima, 1995). Ohshima's soft particle theory is increasingly being employed to interpret electrophoretic mobility data of microorganisms (Kim et al., 2009b; Kim et al., 2010; Liu et al., 2009; Liu et al., 2010), with limitations at lower ionic strength conditions noted for *C. parvum* oocysts (Liu et al., 2009). Attachment of bacteriophages onto clay minerals has been attributed to hydrophobic interactions (Chattopadhyay and Puls, 1999). In contrast, Syngouna and Chrysikopoulos (2010) reported that viruses attach to kaolinite and bentonite clays at the secondary minimum.

While SWIs exist in both saturated and unsaturated soils, the AWI is a salient feature of unsaturated soils. Negatively charged microorganisms will experience repulsive electrostatic and van der Waals interactions at the AWI because the AWI has a negative charge and Hamaker constant (Bradford and Torkzaban, 2008; Schäfer et al., 1998a). Positively charged microbes may therefore adhere to the AWI through electrostatic interactions (Wan and Tokunaga, 2002), whereas negatively charged microorganisms may adhere to the AWI through hydrophobic interactions (Schäfer et al., 1998a; Wan et al., 1994). Hydrophobic forces, dependent on interfacial Lewis acid-base and van der Waals interactions, play important roles in interactions between low energy (hydrophobic) surfaces (van Oss, 1994, 2003; van Oss et al., 1986; van Oss et al., 1987). The AWI is reported to be highly hydrophobic (van Oss et al., 1986; van Oss et al., 1987). Attachment to the AWI and microbe aggregation is therefore expected to increase with the microbe hydrophobicity. Strong capillary forces can greatly contribute to retain pathogens once they interact with the AWI (Bradford and Torkzaban, 2008).

It is sometimes useful to convert the DLVO or XDLVO interaction energies that act to immobilize microbes on the SWI at a separation distance (r , L; where L denotes units of length) into a net pull-off adhesive force (F_A ; $M L T^{-2}$; where M and T denote units of mass and time, respectively) and

a corresponding adhesive or resisting torque ($T_{adhesive}$; $M L^2 T^{-2}$). This information is needed in force and torque balance equations to simulate microbe trajectories and to determine criteria for microbe immobilization and detachment (Bradford et al., 2011; Bergendahl and Grasso, 2000; Duffadar and Davis, 2008). The value of F_A that is required to mobilize a colloid from a primary or secondary minimum in the interaction energy, Φ_{min} [ML^2T^{-2}], can be calculated using the Derjaguin and Langbein approximations (Israelachvili, 1992) that determine F_A as Φ_{min}/r when r is much smaller than the colloid radius, r_c [L]. Only a portion of the projected colloid surface area with the SWI makes a meaningful contribution to F_A (Israelachvili, 1992). This zone of electrostatic influence (Duffadar and Davis, 2007) will produce an adhesive or resisting torque ($T_{adhesive}$; $M L^2 T^{-2}$) and a corresponding frictional force (F_F , $M L T^{-2}$) that acts parallel to the SWI. The values of $T_{adhesive} = l_x F_A$ and $F_F = \mu_f F_A$ where l_x [L] is the lever arm and μ_f [-] is the coefficient of friction (Bergendahl and Grasso, 1998; Lindeburg, 2001). The values of l_x and μ_f are related to each other through $l_x = \mu_f r_c$ (Lindeburg, 2001). Relatively few methods currently exist to estimate l_x in the zone of adhesive influence. Theory by Johnson, Kendall, and Roberts (JKR) has been developed to account for resistance due to deformation (Johnson et al., 1971), and this theory has been used to quantify l_x under favorable (Bergendahl and Grasso, 2000) and unfavorable (Bradford et al., 2007; Torkzaban et al., 2007) conditions for attachment. Alternatively, others have argued that l_x is related to friction that stems from surface roughness so that an empirical value of $\mu_f = 1.3E-4$ was assumed when simulating colloid trajectories over a SWI with positively charged nanoscale heterogeneity (Duffadar and Davis, 2008). It should be mentioned that if $l_x = 0$ then $T_{adhesive}$ and F_F also equal 0. Therefore, no colloid immobilization will occur on the SWI in the presence of fluid flow under favorable or unfavorable conditions (Bradford et al., 2011).

Many bacteria also colonize surfaces. Surface-attached microbial communities, called biofilms, are ubiquitous in aquatic systems, soil and sediment grains, and plant surfaces (Costerton et al., 1995). There is considerable interest in biofilms as it has recently been recognized that most microbial biomass in terrestrial and shallow aquatic systems occurs in the form of biofilms. Additionally, biofilms also frequently foul engineered water systems (e.g., in building water systems, food-handling facilities, and drinking water distribution systems).

Biofilms are dynamic structures that undergo cycles of cell attachment, growth, detachment, and sloughing. Cells in biofilms can exhibit phenotypic differences from planktonic cells because of complex, dynamic patterns of intercellular interaction and signaling. For example, gene expression changes in quorum sensing cells when a minimum threshold of stimulatory concentration of an autoinducer is achieved with an increase in the cell density (Miller and Bassler, 2001; Park et al., 2003). Once in a biofilm, many microorganisms excrete a complex mixture of EPS, which serve to bind cells together

and protect them from predation and harsh environmental conditions (Fleming and Wingender, 2010; Or et al., 2007). The result is a heterogeneous biofilm structure consisting of both cell clusters and interstitial voids (de Beer et al., 1994a; de Beer et al., 1994b). Biofilms found on aquatic surfaces are extremely diverse and accommodate a wide variety of bacteria. Similarly, algal-bacterial assemblages form on surfaces with sufficient illumination to support photosynthesis (Arnon et al., 2007; Barranguet et al., 2005; Battin et al., 2007; Costerton et al., 1995; Rickard et al., 2003). Biofilms are expected to strongly influence waterborne disease transmission because they have been shown to protect resident organisms, including those that are considered pathogenic, from environmental stresses (Costerton et al., 1987; Costerton et al., 1995; Hall-Stoodley et al., 2004; Parsek and Singh, 2003). Biofilms have been implicated in the persistence of pathogens in engineered water systems (Battin et al., 2007; Costerton et al., 1995; Costerton et al., 1999; Hall-Stoodley et al., 2004).

Many fecal zoonotic bacteria are known to form biofilms, including *E. coli* O157:H7, *Salmonella spp.*, and *Campylobacter spp.* (Cotruvo et al., 2004; Dewanti and Wong, 1995; Joshua et al., 2006; Reisner et al., 2006; Rivas et al., 2007). The extracellular matrix produced by biofilms profoundly modifies the properties of colonized environmental surfaces. Colloid deposition onto biofilms has been repeatedly demonstrated in small batch reactors and mesocosms (Drury et al., 1993; Eisenmann et al., 2001; Flood and Ashbolt, 2000; Okabe et al., 1997). Biofilms have also been shown to capture and concentrate protozoan cysts, bacteria, and viruses (Buswell et al., 1998; Mackay et al., 1998; Searcy et al., 2006a; Storey and Ashbolt, 2001). As a result, biofilms are expected to strongly affect the fate of all waterborne pathogens in soils and aquifers, even non-biofilm-forming organisms (Klayman et al., 2009; Liu and Li, 2008; Liu et al., 2008; Wang et al., 2011). Pathogens remobilized from soil or sedimentary biofilms generally occur as cell clusters bound by EPS matrix and contain a wide variety of material that contributes to the mixed organic-inorganic flocs often found in surface waters (Liss et al., 1996; Droppo, 2001). The presence of EPS has also been shown to influence the subsequent transport and deposition behavior of suspended cells and particles (Liu et al., 2008; Tong et al., 2010).

3.2 Pathogen Survival

The survival of pathogens in the environment is normally defined in terms of retaining their ability to infect their host(s). Survival of pathogens and indicators in the environment is dependent upon both the nature of the environment and the organism. Microcosm studies have yielded considerable information about survival under specific sets of well-defined and controlled conditions. These investigations have revealed many factors that affect the survival of pathogens and indicators in agricultural settings. These factors

include, but are not limited to pH, dissolved (DOC) and particulate organic carbon (POC), levels of other nutrients, degree of saturation (moisture content), temperature, ionic strength/salinity, redox conditions, UV exposure, presence/absence of biofilms, environmental microbial diversity and ecology, physicochemical characteristics of the solid phase (mineralogy, grain size distribution, clay content), and nature of the rhizosphere. Agreement on the general knowledge regarding the environmental survival of pathogens and indicators is inhibited by interpretation of data collected from different physicochemical conditions, types of microcosms, methods of measurement, and specific strains. Unfortunately, most survival studies report results for very limited set(s) of conditions. Pathogens traveling from applied manure through the soil and, subsequently, through an underlying aquifer and into water supply wells involves microbial survival in several disparate environments of characteristically different conditions. Consequently, survival throughout the collective transport pathways is exceedingly difficult to predict, resulting in considerable uncertainty concerning survival of pathogens in agricultural settings.

Although there appear to be common factors contributing to the die-off/inactivation of pathogens and indicator organisms, the relative importance of the aforementioned factors can vary considerably with the pathogen of concern. For example, viruses and the zoonotic pathogenic protists of interest in agricultural settings are obligate parasites and, consequently, do not replicate outside their hosts. Conversely, bacterial pathogens are generally capable of growth outside their hosts under favorable conditions. Therefore, it is useful to discuss separately survival of viruses, protists, and bacteria.

VIRUSES

The ability of viruses to infect a host is both time- and condition-dependent. This ability is often lost due to two dominant processes; namely degradation of the viral genome and disruption of the capsid (protein coat surrounding the nucleic acid) (Gerba, 1984). The vast majority of environmentally relevant studies involving virus survival indicate that inactivation is strongly affected by temperature (Schijven and Hassanizadeh, 2002). Higher temperatures can accelerate damage to specific viral components that are required for infection. Most notable are the degradation of the viral genome and conformational changes in proteins associated with the host recognition site (Harvey and Ryan, 2004). However, there is considerable disagreement in the literature on the nature of the temperature-inactivation relationship (John and Rose, 2005).

Not surprisingly, many reports involving survival of viruses in manure indicate strong temperature dependence (Deng and Cliver, 1992b, 1995a; Johnston et al., 2003; Wei et al., 2009; Wei et al., 2010). Studies by Johnston et al. (2003) suggest that coliphage T-1 in manure is capable of surviving during the winter season in cold climate regions. Persistence of viruses in

manure that is applied at higher temperatures is considerably shorter, but still significant. In contrast, Wei et al. (2009) reported near-complete die-off of three human enteric viruses (Murine norovirus 1, Aichi virus, and human adenovirus 41) in composted dairy manure subject to temperatures between 55 and 70°C. The high organic content of manure has been shown to affect viral survival in two counteracting ways. First, organic matter adsorbs onto the viral entity and acts as a protective coating that increases the viral survival rate (Foppen et al., 2006; Pieper et al., 1997; Ryan et al., 2002). Second, the microbial community that thrives in such nutrient rich environments tends to have an antagonistic effect on viruses due to production of proteolytic enzymes, which degrade the viral genome (Deng and Cliver, 1995b). In agreement with bacterial activity, viral persistence in animal wastes has also been shown to depend on the degree of aeration (Pesaro et al., 1995).

Two key processes responsible for natural disinfection of pathogenic and indicator viruses traveling through soils and aquifers are permanent (irreversible) attachment and inactivation. As indicated above, temperature is one fundamental cause for virus inactivation. However, when the subsurface temperatures are $\leq 10^\circ\text{C}$, this type of inactivation would be expected to be minimal and the rate of natural disinfection would depend upon surface-induced inactivation and irreversible attachment. Because transport of pathogens in agricultural settings at environmentally relevant field scales generally involves multiple contacts with solid surfaces, it is important to distinguish between inactivation on a surface and inactivation in solution. The earliest models describing virus transport through porous media assumed that inactivation occurred only in the liquid phase or that inactivation rates of viruses associated with solid surfaces and in the liquid phase were equivalent. However, it has been well established that viral inactivation occurs both in the liquid phase and on solid surfaces (Gerba, 1984) with significantly different rates of inactivation (Grant et al., 1993).

Considerable disagreement remains regarding the effect of virus association with solid surfaces on inactivation rates. Discrepancies in reported experimental observations are likely due to the use of different viruses (John and Rose, 2005) and different experimental conditions (e.g., media of different characteristics) for each study (Zhao et al., 2008). In general, viral inactivation rates tend to be diminished by sorption onto geologic media of high clay content and organic matter (Liew and Gerba, 1980; Stagg et al., 1977; Straub et al., 1993). Conversely, viral inactivation is enhanced by sorption onto iron and aluminum oxide minerals that bind strongly the virus onto the medium's surface (Blanc and Nasser, 1996; Chu et al., 2003; Ryan et al., 2002; Schijven et al., 1999). For injection and recovery studies involving virus transport through granular aquifers containing iron- and aluminum-oxides, inactivation of sorbed viruses was greater than that for unattached viruses (Bradford et al., 2006d; Chu et al., 2001; Flynn et al., 2004; Pieper et al., 1997; Ryan et al., 2002; Schijven et al., 1999). The presence of dissolved metal ions

has also been reported to enhance virus inactivation in water (Sagripanti et al., 1993).

Harvey and Ryan (2004) proposed that virus inactivation may be caused by the strong interactive forces characterizing negatively charged viruses with spatially and/or temporally variable positively charged metal oxide surfaces. An enigma in the surface-inactivation concept is that mineral surfaces that exhibit strong net positive charge under mildly acid to neutral conditions (e.g., iron and aluminum oxides) are expected to irreversibly retain the viruses (Loveland et al., 1996). However, release of viruses (both intact and disaggregated) has been observed for these types of surfaces (Murray and Laband, 1979; Ryan et al., 1999). One possible explanation is due to spatial and/or temporal variability in the strength of virus interactions with the SWI that influence both virus attachment and inactivation.

In general, viral inactivation in soils and groundwater is decreased by natural and contaminant-derived organic carbon (e.g., Foppen et al., 2006; Ryan et al., 2002; Sakoda et al., 1997). It has been proposed that the role of organic matter in both the attachment and inactivation of viruses in the subsurface can be quite complex (Schijven and Hassanizadeh, 2000). Decay rates for some viruses have been shown to be lower in wastewater than in groundwater incubated at comparable temperature (Nasser and Oman, 1999). For field injection and recovery studies involving transport of indicator virus PRD1 through iron-containing aquifer sediments, the presence of waste-derived organic matter decreased partitioning of the virus to the iron-containing sand grains (Blanford et al., 2005; Pieper et al., 1997; Ryan et al., 1999); thereby enhancing survival and transport. Schijven et al. (1999) found that inactivation of MS2 and PRD1 (bacteriophages) were increased by 34-fold when native groundwater was used as the liquid medium compared with a saline solution containing peptone.

In most reported studies, virus survival decreased in response to decreased soil moisture content, particularly when soil moisture dropped below 5% (Song et al., 2005; Straub et al., 1992; Straub et al., 1993). Nonetheless, exceptions to this phenomenon have been reported by various studies (e.g., Davies et al., 2006). Song et al. (2005) suggested the existence of optimal range of combined temperatures and soil moisture contents that permit virus survival in soils. Zhuang and Jin (2003) reported that increasing hydrophobicity of the virus resulted in increased irreversible sorption at the AWI, presumably due to hydrophobic and capillary forces. Thompsom and Yates (1999) reported that virus inactivation was enhanced at the air-water-solid (AWS) triple point due to interfacial forces, which damaged the protein capsid. However, Zhao et al. (2008) indicated that discussion on the effect of soil water content on virus adsorption/inactivation remains largely speculative.

Virus survival in groundwater has been shown to be strongly affected by the level of dissolved oxygen (Gordon and Toze, 2003). However, survival differences often disappear when groundwater is filtered. Therefore,

the role of dissolved oxygen effect is likely an indirect effect involving the antagonistic activities toward the viruses by native microbial communities. A few studies suggest that indigenous bacteria can cause increases in inactivation rates of pathogenic viruses. For example, Deng and Cliver (1992b) showed that several bacterial cultures isolated from swine manure inhibited poliovirus type 1 in waste slurries. The inhibitory effect was attributed to proteolytic enzymes produced by the bacteria. Similarly, it was reported that inhibition of aerobic respiration lessened inactivation of coliphage in soils, resulting in significantly less efficient coliphage attenuation ($p = .033$). This suggests that aerobic bacteria were involved in viral inactivation (Quanrud et al., 2003). Nasser et al. (2002) found that the ability of bacteria to inactivate viruses depended upon the virus type.

PROTISTS

Cryptosporidium parvum and *Giardia duodenalis* infect both humans and domestic livestock and are responsible for a number of waterborne disease outbreaks in the United States and elsewhere (Olson et al., 1999). Prevalence of these pathogens can be as high as 100% in dairy calves, and the feces from infected animals can harbor up to 10^9 *Giardia* cysts and 10^6 *Cryptosporidium* oocysts per gram (O'Handley et al., 1999). Conversely, the infectious dose for these organisms can be quite low, for example just 10–1000 oocysts for infection of humans by *C. parvum* from cattle (Okhuysen et al., 1999). *C. parvum* oocysts can remain infective in livestock wastes for months (Jenkins et al., 1997) and seem to be considerably more persistent than *E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella*, and *Campylobacter jejuni* (Hutchison et al., 2005). Consequently, manure can act as long-term reservoirs for oocysts.

Temperature appears to be the most important factor controlling the persistence of protozoan pathogens from animal waste (Jenkins et al., 1999; Robertson et al., 1992). From a comprehensive survey of the literature, Peng et al. (2008) reported a collective exponential increase in the decay rate of *C. parvum* oocysts in animal feces with increasing temperature, which is the same factor determining persistence of *Giardia* cysts (Olson et al., 1999). The time required to reduce by one log unit the abundance of viable *Giardia* cysts inoculated into a mixture of human and swine manure was reported to be 130 days at 5°C and reduced to four days when temperatures were raised to 25°C (Deng and Cliver, 1992a). Composting of manure has been found to sharply reduce the survival of both *C. parvum* oocysts and *Giardia* cysts once temperature reaches 55°C (van Herk et al., 2004).

Inactivation rates for *Cryptosporidium* oocysts suspended in groundwater collected from Florida and incubated at 4°C were reported to range from 0.0009 to 0.0088 d⁻¹ (Ives et al., 2007). Inactivation appears to be more rapid in soils than in groundwater. For example, inactivation rates for oocysts in soil ranged from 0.005 to 0.027 d⁻¹ at 4°C (Davies et al., 2005; Jenkins et al.,

2002; Olson et al., 1999). *Giardia* cysts have been shown to survive up to 11 weeks in water at 4°C, but were completely inactivated within 1 week in soil at 25°C regardless of whether the soil was autoclaved or not (Olson et al., 1999). Conversely, oocysts remain infective for longer periods of time in autoclaved versus nonautoclaved soil (Olson et al., 1999), suggesting that biotic factors had a strong influence on oocyst survival.

BACTERIA

Manure can harbor a number of zoonotic bacterial pathogens, including *Campylobacter* spp., *Chlamydia* spp., *E. coli*, *Leptospira* spp., *Listeria monocytogenes*, *Mycobacterium* spp., *Salmonella* spp., and *Yersinia* spp. (Ziemer et al., 2010), many of which have been shown to survive for considerable periods of time in manure environments (Johnston et al., 2003; Lau and Ingham, 2001; Williams et al., 2007). Great variability in survival has been reported in different studies, likely as a result of differences in bacterial strains, temperature, and water and nutrient levels. The survival of *E. coli* O157:H7 in feces and soils subject to application of manure has been observed to vary from days to months (Duffy, 2003; Shere et al., 1998; Zhao et al., 1995). Williams et al. (2007) reported that *E. coli* O157:H7 can survive for 5 weeks in cattle wastes applied to soil cores regardless of the presence or absence of a rhizosphere. In another study involving soil amended with bovine manure, it was shown that both *E. coli* and *enterococci* could survive up to 19 weeks at temperatures of up to 21°C (Lau and Ingham, 2001), indicating that low-temperature animal waste treatments (e.g., anaerobic digesters, drying, no treatment) may inactivate pathogen less effectively than high-temperature treatments (e.g., composting, high-temperature fermentation). Survival rates of over one year have been observed for *E. coli*, *Salmonella*, *Campylobacter* spp., and *Enterococcus* spp. in manure slurry, soils receiving manure, pastures, and feedyards (Hutchison et al., 2005; Jones, 1986; Purdy et al., 2001).

Long-term survival has also been shown for some pathogenic and indicator bacteria in agricultural soils. For example, Lang and Smith (2007) have observed that the time required for a log unit reduction for *E. coli* was 100 days and 200 days in a dry sandy loam soil and a silty clay soil, respectively. The survival of microorganisms in soil is due to a complex combination of abiotic and biotic factors that may not always be independent, although a key recurring parameter is temperature. Most studies involving the survival of pathogens and indicators in agricultural soils report the longest persistence at lower incubation temperatures and higher moisture contents (e.g., Cools et al., 2001). Analysis of a large number of reported die-off studies of *E. coli* reveal that the increases in die-off rates per degree (°C) rise are comparable in most experiments (Foppen and Schijven, 2006). Other studies propose that the effect of temperature fluctuations on die-off may be dampened by finer soil textures (Cools et al., 2001) or by the presence and

type of organic carbon (Garzio-Hadzick et al., 2010; Ishii et al., 2010; Vidovic et al., 2007).

It is well accepted that prolonged dry periods or exceptionally low moisture contents affect viability of indicator bacteria such as *E. coli* (Berry and Miller, 2005; Habteselassie et al., 2008; Ishii et al., 2010; Vidovic et al., 2007). A monotonically increasing relationship between water potential and bacterial respiration rate (as a surrogate measurement for viability) was reported by Bazin et al. (1976). Conversely, another study (Halverson et al., 2000) observed dilution stress responses to affect culturability even though sudden increases in soil water potential did not cause significant cell lysis.

The effects of ionic strength and pH on survival are dependent on the microbial species. For instance, halophiles and some salt tolerant bacteria thrive in saline environments because they have the capability to osmoregulate (Essendoubi et al., 2007; Zahran, 1997), whereas other nonhalophilic species typically experience growth arrest and may even go into osmotic shock (Wood and Sørensen, 1998). An early study by Singleton et al. (1982) determined that in the presence of saline conditions the growth of all the species and strains of *Rhizobium* decreased when the electric conductivity of the medium was raised from 1.2 to 6.7 mS cm⁻¹ (specific conductivity) and several strains were completely inhibited at 13.1 mS cm⁻¹. Perhaps more importantly, this study indicates that the effects of salinity on viable count reduction can be delayed for weeks. The effect of pH on bacterial inactivation is lowest at pH levels between 6 and 8 (Foppen and Schijven, 2006) and greatest in both acidic (Inglis and Sagripanti, 2006) and alkaline (Stimson et al., 2010) conditions.

Even when conditions favor the rapid death of the bulk population of pathogens, some sub-populations can persist for a very long period of time. A number of bacterial pathogens and indicators, including *Campylobacter spp.*, *E. coli*, *Francisella tularensis*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa*, *Salmonella spp.*, *Shigella spp.*, and *Vibrio spp.*, appear to be able to enhance their survival in the environment by entering a pseudo-dormant state. Such a slow-growing, persistent state has been termed viable but nonculturable (VBNC; Oliver, 2010), or, in the clinical microbiology literature, as persister cells or small-colony variants (Kussell et al., 2005; Proctor et al., 2006). All of these states can generally be classified as persistent phenotypes, and are adaptations to survival under variable environmental conditions (Kussell et al., 2005; Rotem et al., 2010). The VBNC state can be triggered by a variety of environmental factors, including: nutrient deprivation (Cook and Bolster, 2007); changes in temperature (Besnard et al., 2002); oxygen levels (Kana et al., 2008); salinity (Asakura et al., 2008); and presence of heavy metals (Ghezzi and Steck, 1999). Cook and Bolster (2007) showed that *E. coli* and *C. jejuni* in groundwater transitioned into a VBNC state characterized by changes in morphology and a reduced rate of respiration in response to starvation and

decreased temperature. Although pathogens cannot cause infection while in the VBNC state, there is evidence to suggest that their virulence is retained (Oliver, 2010), therefore they remain a public health risk. Because most studies involving inactivation and survival of zoonotic bacterial pathogens in agricultural settings employ culture-based methods, the ability of bacterial pathogens to transition into and out of the VBNC state suggests that many of the published environmental persistence/inactivation data may substantively underestimate survival. Persistent phenotypes also often show other differences from the original pathogen population, such as in biochemistry and morphology (Proctor et al., 2006; Thomas et al., 2002), suggesting that they could also have distinct environmental transmission behavior. However, these properties are not well characterized for most zoonotic pathogens, and the net implications of these persistent states for environmental transmission remain unknown.

Survival of bacterial pathogens and indicators in soils can be affected by genetic or intrapopulation variability. A number of studies indicate the presence of subpopulations that are more or less persistent under environmental conditions. Binnerup et al. (1993) found that >99% of DF57-3 cells inoculated into soil microcosms lost their culturability after 40 days. However, ~20% were in a VBNC state and still capable of cell division as evidenced by their ability to form minute microcolonies that escape detection using conventional methods of colony detection. Intrapopulation variability is also evident in the ability of some bacteria (*Bacillus spp.*) to form dormant but robust spores, a strategy used to enhance persistence under starvation conditions (Morohashi et al., 2007).

The activities of indigenous microorganisms in soils and groundwater can substantially enhance or inhibit the survival of zoonotic pathogens and indicators in soils and groundwater. Biofilm formation has been shown to favor the survival of pathogens under both typical environmental conditions and under active disinfection (Buswell et al., 1998; Dykes et al., 2003; Gibson et al., 2006; Joseph et al., 2001; Korber et al., 1997; Latasa et al., 2005; Murphy et al., 2006; Ryu and Beuchat, 2005). Conversely, competition of bacteria species for limited growth substrates and nutrients is well known to inhibit pathogen survival (Crane and Moore, 1986; van Veen et al., 1997). Phages are abundant and ubiquitous in nature (Kimura et al., 2008; Marsh and Wellington, 1994) and are therefore important components of microbial communities. They can impact host bacterial populations by predation and altering the host phenotype by genetic interactions (Dröge et al., 1999; Marsh and Wellington, 1994). It is likely that coliphages may play an antagonistic role in the survival of coliform bacteria in agricultural settings. The role of protozoa in the survival of bacterial pathogens in the subsurface is not well understood. However, it is known that the diverse communities of nanoflagellates in aquifers (Novarino et al., 1997) can be a significant sink for bacteria during advection through aquifer sediments (Kinner et al., 1997;

Kinner et al., 1998). The role of predation appears to increase where there are substantive inputs of DOC (Kinner et al., 2002) because the protistan community is stimulated by bacterial growth. Conversely, some bacterial pathogens can prolong their survival in subsurface environments by residing in and multiplying within certain protozoa. For example, it has been shown that *E. coli* O157 can reside and replicate in *Acanthamoeba polyphaga* in soils (Barker et al., 1999).

The ability of bacterial pathogens to grow in the environment has been a topic of ongoing research, as growth depends on the conditions and the specific pathogen of interest. Some (e.g., *E. coli* and *C. jejuni*) are normal inhabitants of soil environments and are expected to proliferate in-situ under favorable conditions (e.g., optimal temperature, nutrients, substrates, water content, and lack of competition and predation). Growth in the environment of nonindigenous bacteria of public health interest is often referred to as regrowth (Zaleski et al., 2005a), as it is suspected to occur if the optimal growth conditions are met. For example, *E. coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes* inoculated separately into autoclaved compost were observed to increase in abundance by 2.1–3.9 log within 3 days (Kim and Jiang, 2010). In another study, increases (of up to 1.5 orders of magnitude) were observed in *enterococci*, *E. coli*, fecal streptococci, and *S. enterica* in cow manure over the first 1–3 weeks of incubation (Sinton et al., 2007). Lau and Ingram (2001) reported that *E. coli* abundance initially increased 1–2 log units in soil amended with bovine manure. Zaleski et al. (2005b) reviewed available information on the possibilities of regrowth of pathogenic bacteria and indicators in manure, compost, soil, and manure-amended soil and concluded that regrowth of indicators and *Salmonella* is possible in biosolids under conditions of favorable moisture and temperature, or substrate availability. However, Zaleski et al. (2005b) also noted that indigenous microorganisms were important in controlling potential regrowth and that storage of biosolids can be effective in reducing pathogen levels at favorable moisture and temperature conditions.

PATHOGEN SURVIVAL CURVES

The combined influence of die-off and growth leads to many different types of pathogen survival behavior in agricultural settings. Crane and Moore (1986) reviewed various pathogen survival curves that are presented in Figure 1. Curve 1 is characteristic of simple, constant, first order die-off. In curve 2 a lag period exists before constant, first-order decay. This lag period may be caused by a reduction of environmental stresses due to dilution (e.g., lowering of toxic compounds, increased nutrient supply, and/or favorable changes in the oxygen availability). Curve 3 is characteristic of a constant reduction in the survival rate with time as a result of differences in the survival behavior of the population. In curve 4 the decay rate is increasing with time in a nonlinear manner. This may reflect the influence of an

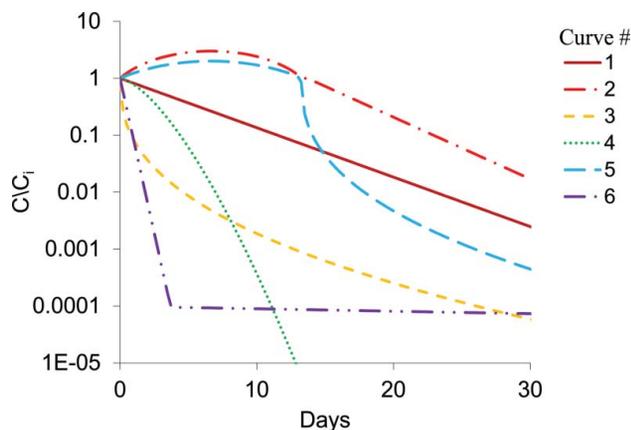


FIGURE 1. Illustrative survival curves for microorganisms in soil based on literature information (adapted from Crane and Moore, 1986). Here C is the microbe concentration, and C_i is the initial microbe concentration. Curve 1 is for simple, constant, first order die-off. In curve 2 a lag period exists before constant, first-order decay. Curve 3 is due to a constant reduction in the survival rate with time. In curve 4 the decay rate is increasing with time in a nonlinear manner. Curve 5 reflects an initial growth period followed by a time dependent decay term. Curve 6 reflects a high initial death rate followed by a slower rate of die-off (Color figure available online).

increasing concentration of toxic compounds on survival. Curve 5 reflects an initial stationary or growth period followed by a time dependent decay term. Finally, curve 6 reflects a high initial death rate due to shock, such as when microorganisms are initially introduced into a new environment, followed by a slower rate of die-off after acclimatization.

In sum, pathogens are known to exhibit a wide variety of behavior in manure, soil, and sedimentary environments. Simple, first-order die-off should not generally be assumed, particularly as most soil and subsurface environments are highly heterogeneous, as described in previous sections. The potential for long-term survival of persistent subpopulations and regrowth in the environment are particular concerns for pathogen transmission. However, available information is insufficient to draw general conclusions about the behavior of most important classes of pathogens found in soil environments. More research is needed on the properties of persistent subpopulations, transformations between active and vegetative (e.g., persistent or nonculturable) phenotypes, interactions between fecal pathogens and indigenous microbial populations, and factors that control the regrowth of pathogens in the environment.

3.3 Pathogen Transport and Retention at the Pore Scale

Figure 2 presents an illustration of various processes influencing pathogen transport at the pore scale that will be discussed in detail below.

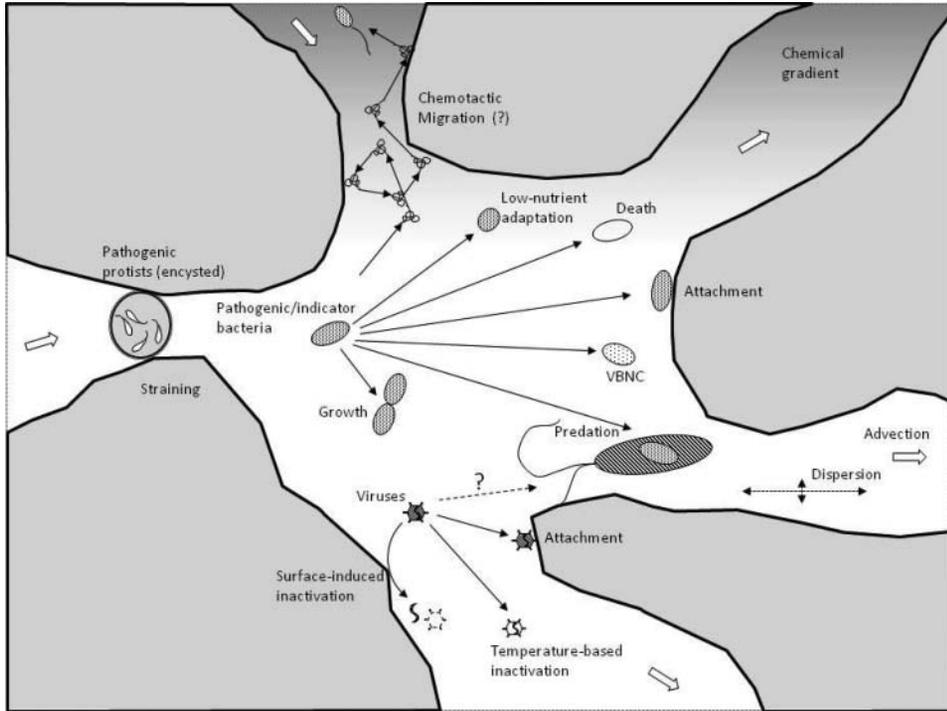


FIGURE 2. An illustration of various processes influencing pathogen transport at the pore scale.

The mass transfer of pathogens to the various interfaces that exist in soil pores (e.g., SWI and AWI) need to be properly accounted for in order to effectively describe the microbe retention. Colloid filtration theory (CFT), designed for saturated porous media, considers that retention is a two-step process where (a) a fraction of the colloid flux collides with the SWI, represented by the single-collector contact efficiency (η); and (b) a fraction of colloids that experienced collision with the SWI will become retained, represented by the attachment efficiency (α) (Rajagopalan and Tien, 1976; Tufenkji and Elimelech, 2004a; Yao et al., 1971). The parameter η is defined as the ratio of the integral of the colloid flux that strikes the collector to the rate at which particles flow toward the collector (Yao et al., 1971). The value of η has been determined by solution of Stokes flow, continuity, and convective diffusion or trajectory equations for ideal single collector systems to determine the mass flux due to interception, sedimentation, and diffusion. Filtration theory was originally developed using a single spherical collector in the Happel sphere-in-cell model (Happel, 1958; Payatakes et al., 1974). The overall porosity of a porous medium is accounted for in this model by adjusting the thickness of a continuous liquid sheath surrounding the collector surface. Correlation equations to predict η as a function

of system variables (water velocity, colloid size and density, and collector size) have been developed from simulation results (Rajagopalan and Tien, 1976; Tufenkji and Elimelech, 2004a; Yao et al., 1971). More recently, the sensitivity of η to variations in collector shape and roughness was found to be significant (Saiers and Ryan, 2005). Correlation equations have also been developed to estimate η using a hemispheres-in-cell model in saturated media that preserves the grain-grain contact point (Ma and Johnson, 2009; Ma et al., 2009). It should be mentioned, however, that the correlations developed for η are only explicitly valid for saturated systems that consider only a single grain size. Mathematical expressions to describe colloid filtration for a distribution of grain sizes and in unsaturated media are still unresolved. In unsaturated systems two complications must be dealt with (a) the presence of an air phase that complicates the fluid shell geometry; and (b) the presence of AWIs and AWS contact lines that can act as sinks for microbes (Crist et al., 2004; Crist et al., 2005; Sirivithayapakorn and Keller, 2003; Wan and Wilson, 1994a, 1994b; Zevi et al., 2005).

Filtration theory was originally developed for favorable attachment conditions ($\alpha = 1$). Several theoretical approaches have been developed to estimate α under unfavorable attachment conditions (Dong et al., 2002; Elimelech et al., 2000; Shen et al., 2007; Simoni et al., 1998; Spielman and Friedlander, 1974). Some researchers have related α to the fraction of the solid surface area that is chemically favorable for deposition (Elimelech et al., 2000), such as the case of iron oxyhydroxide patches on quartz grains. Others have related α to the probability to diffuse over of the energy barrier into the primary minimum (Spielman and Friedlander, 1974). More recently, attempts have been made to divide α into contributions from the primary (α_1) and secondary (α_2) minimum. These two types of interaction energetically account for deposition of small (nano sized) and large (micron sized) colloids, respectively (i.e., $\alpha = \alpha_1 + \alpha_2$). The kinetic energy method assumes that a particle's kinetic energy can be described by a Maxwell distribution function and that DLVO or XDLVO energy profiles accurately predict the interaction energies between particles and the collector such that α_1 and α_2 are determined as (Shen et al., 2007; Simoni et al., 1998):

$$\alpha_1 = \int_{\Phi_{2\min} + \Phi_{bar}}^{\infty} \frac{2\sqrt{\Phi}}{\sqrt{\pi}} \exp(-\Phi) d\Phi \quad (1)$$

$$\alpha_2 = \int_0^{\Phi_{2\min}} \frac{2\sqrt{\Phi}}{\sqrt{\pi}} \exp(-\Phi) d\Phi \quad (2)$$

where Φ [–] is the kinetic energy of the colloids, $\Phi_{2\min}$ [–] is the magnitude of the dimensionless (divided by the product of the Boltzmann constant and the absolute temperature) depth of the secondary energy minimum, and Φ_{bar}

[–] is the dimensionless height of the energy barrier. This analysis indicates that a fraction of colloids will possess sufficient kinetic energy to detachment from the SWI at any time that is given by $1-\alpha$. Equations [1] and [2] could similarly be used to determine α on the AWI if the interaction energy between the AWI and the colloids is known a priori (Zhang et al., 2010b). However, in this case additional interactions would likely need to be considered such as hydrophobic and capillary forces (Bradford and Torkzaban, 2008).

It is important to recognize that Equation [1] overestimates α_1 because colloids with sufficient kinetic energy to escape the secondary minimum and the energy barrier may also escape primary minimum retention and re-enter the bulk suspension. Equation [2] is of particular importance in the mathematical justification of weak and irreversible colloid retention in the secondary energy minimum. However, it should be mentioned that it is possible that the value of α_2 may be a function of velocity (Johnson et al., 2007c; Torkzaban et al., 2007) and input colloid concentration (Chapman and Cowling, 1991), and these factors are not considered in the kinetic energy method.

CFT assumes that colloids are immobilized upon contact with the SWI and neglects the role of the pore structure in colloid retention. Recent research has demonstrated that weakly associated colloids with the SWI and/or AWI may experience significant hydrodynamic forces due to fluid flow that may result in rolling, sliding, skipping, or detachment of colloids on/from the SWI and/or AWI (Bergendahl and Grasso, 1998, 1999; Bradford and Torkzaban, 2008; Duffadar and Davis, 2008; Hubbe, 1984; Kuznar and Elimelech, 2007; Torkzaban et al., 2007; Torkzaban et al., 2008b; Tsai et al., 1991). Some of these weakly associated colloids can be translated and/or funneled by fluid drag forces to regions that are associated with greater adhesive forces (chemical heterogeneity) or lower fluid velocities where they can be immobilized (Bradford and Torkzaban, 2008; Lazouskaya and Jin, 2008; Torkzaban et al., 2007; Torkzaban et al., 2008b; Zevi et al., 2012). Conversely, the applied hydrodynamic torque that a pathogen experiences on the SWI, and which can result in detachment is expected to be greater with pathogen size and Darcy velocity, and smaller with collector size and porosity (Bradford et al., 2011).

The pore structure controls the presence of low velocity regions in porous media. In saturated porous media, low velocity vortices or eddy zones can occur at grain-grain contacts, in pore throats and over surface roughness (Cakmak et al., 2012; Cardenas, 2008; Li et al., 2010a; Li et al., 2010b; Sheng and Zhou, 1992; Taneda, 1979; Torkzaban et al., 2008b). Under unsaturated conditions, the fluid distribution controls the presence of low velocity regions that occur near the AWS contact line and in thin water films. These locations have also been associated with hydrophobic and/or capillary forces (Gao et al., 2006; Gao et al., 2008; Lazouskaya and Jin, 2008; Zevi et al., 2012;

Zhang et al., 2010b). Microscopic observations have confirmed that enhanced colloid retention occurs in such low velocity regions of the porous media under unfavorable attachment conditions (Bradford et al., 2005; Bradford et al., 2006a; Bradford et al., 2006b; Choi et al., 2007; Crist et al., 2004; Crist et al., 2005; Gaillard et al., 2007; Gao et al., 2006; Lazouskaya and Jin, 2008; Li et al., 2006a, 2006b; Tong et al., 2008; Xu et al., 2006; Yoon et al., 2006; Zevi et al., 2005; Zevi et al., 2009; Zevi et al., 2012; Zhang et al., 2010b).

Various terms for colloid retention at the pore scale have been applied in the literature. In brief, colloid retention on a single interface (SWI or AWI) has been referred to as attachment. Retention of colloids near two or more bounding interfaces has been referred to as straining (Bradford et al., 2002; Bradford et al., 2006a; Cushing and Lawler, 1998; Hill, 1957), wedging (Herzig et al., 1970; Johnson et al., 2007b), bridging (Ramachandran and Fogler, 1999), film straining (Wan and Tokunaga, 1997), or retention at the AWS triple point (Chen and Flury, 2005; Crist et al., 2004; Crist et al., 2005). Bradford and Torkzaban (2008) noted that colloid retention at multiple interfaces share many similarities—namely low-velocity regions—and can all be encompassed by the more general definition of straining as colloid retention in the smallest regions of the pore space. When all of the pore spaces in a porous medium are smaller than the colloid diameter, then complete retention of colloids occurs via mechanical filtration (McDowell-Boyer et al., 1986).

Pronounced differences in transport and fate are expected for free, aggregated, and/or particle-associated pathogens. In particular, rates of liquid and solid phase (rolling) mass transfer, the strength of chemical interactions, and the ability of pathogens to move through complex pore geometries will be influenced by the degree of pathogen-particle associations. The partitioning of microbes between free and particle-associated states will depend greatly on aggregation/disaggregation kinetics. Factors that influence aggregation, as was discussed in the preceding sections, include chemical interactions (pH, ionic strength, solution composition, charge, and surface macromolecules), particle size distribution and particle density. Aggregation of microorganisms by nucleation growth may also occur as a result of retention in grain-grain contacts (Bradford et al., 2006b; Tong et al., 2008), dissolution of the gas phase (Sirivithayapakorn and Keller, 2003), and hydrophobic interactions (Lazouskaya et al., 2006). Cementation of cell aggregates occurs similarly to biofilm formation (Voloshin and Kaprelyants, 2004).

Bacterial extracellular appendages or surface structures such as flagella, pili, curli, and polysaccharides are suspected to also play a key role in bacterial transport and retention through soils (Stevik et al., 2004). Some extracellular structures like lipopolysaccharides, curli and pili are typically associated with strains that are easily retained in soils, as it is hypothesized that these small appendages help cells hold onto surfaces (Rijnaarts et al.,

1995; van Houdt and Michiels, 2005; Walker et al., 2004). In contrast, extracellular structures like flagella and cilia are primarily mechanisms of cell motility in suspension and are usually associated with mobile strains that favor nomadic lifestyles (Kolter and Greenberg, 2006).

It is well recognized that certain microorganisms in aquatic environments are capable of moving (Camper et al., 1993; Schneide and Doetsch, 1974). Although maximum bacterial swimming speeds of over $400 \mu\text{m s}^{-1}$ have been observed, macroscopic migration rates are considerably slower. Also, nutrient gradients are highly transitory because motile bacteria periodically change swimming directions (Barbara and Mitchell, 2003). It is also important to note that the presence of a porous medium reduces the cell motility due to the longer paths required to move around impenetrable solids. Harshey (2003) describes the mechanisms used for motility as follows: swimming and tumbling (single cell) and swarming (colonial) are dependent on the presence of flagella to propel cells through the liquid medium, whereas twitching and some forms of gliding on surfaces require active extension and retraction of type IV pili. In addition to classical run-and-tumble motility, a number of other types have been identified, including run and stop, run and reverse, and run and arc (helical klinotaxis; Mitchell and Kogure, 2006). Sliding, spreading, and swarming on surfaces, often facilitated by release of surfactants, play a significant role in bacterial surface colonization. Studies that have investigated motility with respect to bacterial transport reported that the cell's ability to swim did not hasten breakthrough of microorganisms (Camper et al., 1993). However, attachment rates for motile bacteria have sometimes been reported to be greater than nonmotile bacteria (McClaine and Ford, 2002; Mueller, 1996), whereas others have found contradictory or inconclusive results (Becker et al., 2004; Camesano and Logan, 1998). Additional research is warranted to investigate the influence of unsaturated conditions on cell motility.

Chemotaxis is the self-generated displacement of motile microbes in the direction of an increasing concentration gradient of a chemo-attractant or a decreasing concentration gradient of a chemo-repellent. Physical and chemical signals that initiate motility toward more favorable micro-environments include: moisture availability, nutrients, slime, and temperature. The interested reader is referred to Ford and Harvey (2007) for a review of the role of chemotaxis in bacterial transport through saturated granular media.

3.4 Pathogen Transport and Retention at the Column Scale

Pathogen transport and retention at the column scale is a function of many physicochemical properties of the system, including: chemistry of the soil, solution, and pathogen; pathogen size and concentration; system hydrodynamics; pore-space geometry; and water content. Filtration theory (Yao

et al., 1971) attempts to account for many of these factors by estimating the attachment rate (k_{asw} , T^{-1}) in saturated porous media as

$$k_{asw} = \frac{3(1 - \theta)}{2d_{50}} \alpha \eta v \quad (3)$$

Here v [LT^{-1}] is the average pore water velocity, θ is the volumetric water content, and d_{50} [L] is the median grain diameter. The theoretical determination of η was discussed earlier. The value of η is a function of the pathogen size and density, the pore water velocity, and the collector size. The buoyant density of bacteria and protozoa transported into an aquifer can be altered, depending upon the amount of time spent under in-situ nutrient conditions. For example, it has been shown for some bacteria and protists that growth under lower nutrient conditions may lead to smaller cells having lower specific gravities (Harvey et al., 1997; Harvey et al., 2002). A nonlinear dependence of η on pathogen size is predicted with filtration theory, with a minimum value for η occurring when the colloid radius approaches 1 μm . Exceptions to this critical size have been found to depend in part on the buoyant density of the microorganism (Harvey and Garabedian, 1991). The value of k_{asw} also increases with decreasing d_{50} , and is proportional to v raised to the 1/3 power (Schijven and Hassanizadeh, 2000). However, the overall rate of advection is proportional to v such that a systematic decrease in retention is expected with an increase in velocity.

Filtration theory originally assumed that colloids/microbes were irreversibly retained in the primary minimum of the DLVO interaction energy distribution (Ryan and Elimelech, 1996). More recently, however, attachment and re-entrainment of pathogens in laboratory (Harter et al., 2000; Johnson et al., 2007a; Loveland et al., 1996; Scholl and Harvey, 1992) and field studies (Schijven et al., 1999; Zhang et al., 2001a) have been attributed to reversible secondary minimum interactions. The chemistry of the pathogen, solution, and solid phase will have a strong influence on the interaction energy profile (height of the energy barrier and depth of the secondary minimum), and therefore affect α . Numerous studies have demonstrated that retention of negatively charged colloids, including most cells, is enhanced in porous media when the solution chemistry is at a lower pH, higher IS, or contains higher concentrations of divalent cations because, as discussed above, these conditions generate favorable conditions for attachment (e.g., Ryan and Elimelech, 1996).

Under unfavorable attachment conditions it is possible to have localized regions that are favorable for attachment (e.g., geochemical heterogeneity), thus requiring for the value of α to be proportional to the fraction of the solid surface area that is favorable for attachment (Abudalo et al., 2005; Elimelech

et al., 2000). Geochemical heterogeneity involves spatial variations in a number of properties of the solid phase, including mineralogy, grain surface irregularities of metal oxides, and positively charged clay content (Harvey and Ryan, 2004). Harvey et al. (1993) found in a sandy unconfined glaciofluvial aquifer located in Cape Cod, MA that bacteria breakthrough were retarded due to deposition in layers or pockets of fine grain sediments and/or due to sorption onto metal oxide coated grain surfaces of the aquifer at different depths. Recent studies have shown a positive correlation between the charge density on iron oxide coated sand to their filtration capacity of oocysts and viruses (Abudalo et al., 2005). Soils rich in clay minerals and metal oxides have been shown to sorb viruses and oocysts irreversibly (Chu et al., 2003; Mohanram et al., 2010; Zhang et al., 2010a; Zhao et al., 2008). The type, abundance, and surface coverage of extractable iron and aluminum oxides on the grains have been shown to be a major factor controlling transport of viruses (Pieper et al., 1997; Ryan et al., 1999), bacteria (Mills et al., 1994; Foppen et al., 2008), and protozoa (Abudalo et al., 2005; Metge et al., 2011). Viral and bacterial retention in clay-rich soils have been positively correlated to the soil's clay content (Lipson and Stotzky, 1983; Huysman and Verstraete, 1993b). Other studies have found that *E. coli* and *Pseudomonas putida* attached more to kaolinite clays than montmorillonite clays (Huysman and Verstraete, 1993a; Jiang et al., 2007). In addition, the sedimentation velocity for oocysts increased by a factor of 50 when associated with inorganic particles such as kaolinite clays or natural inorganic-organic aggregates (Searcy et al., 2005). A sensitivity analysis by Sun et al. (2001) demonstrated that favorable (fast) and unfavorable (slow) deposition coefficients and geochemical heterogeneity were highly interrelated in 2-dimensional simulations. The above information demonstrates that geochemical heterogeneity in the form of spatial variability in the amounts of iron and aluminum associated with grain surfaces or the type and distribution of clays is an important parameter in determining the extent of microbial transport in granular media.

The presence of dissolved organic matter (DOM), surfactants, and disaggregated manure have been shown to significantly reduce the role of geochemical heterogeneity and thereby enhance microbe transport through granular media (Abudalo et al., 2010; Bradford et al., 2006c; Bradford et al., 2006d; Brown and Jaffé, 2001; Cao et al., 2010; Foppen et al., 2006; Guber et al., 2005a; Guber et al., 2005b; Harvey et al., 2010; Johnson and Logan, 1996; Metge et al., 2010; Pieper et al., 1997; Powelson and Mills, 2001). Natural organic matter (NOM) has been demonstrated to sorb onto metal oxide surfaces (Munn and Spackman, 1990; Tombácz et al., 2001) and clay minerals (Gao et al., 2004a) and to neutralize positively charged sites. Blocking of favorable attachment sites by organic matter has therefore been implicated to explain this enhanced transport (Johnson and Logan, 1996; Moore et al., 1981; Pieper et al., 1997; Zhuang and Jin, 2003; Guber et al., 2005a; Guber et al., 2005b). Filling of straining sites by NOM has been proposed as

an alternative explanation (Bradford et al., 2006c). DOM has also been reported to sorb onto bacterial cell walls and alter their electrophoretic mobility (Gerritsen and Bradley, 1987) and hydrophobicity (Dai and Hozalski, 2003; Harvey et al., 2011; Mohanram et al., 2012). Sharma et al. (1985) reported that DOM increases the negative charge of the microorganism surface, which tends to diminish its attachment onto negatively charged solid surfaces. A recent study by Morales et al. (2011) indicates that in addition to providing a more negative surface charge, DOM affects colloid transport by endowing the suspension with a polymeric shell that generates key steric forces that can enhance transport when uniformly blanketing the colloid, or hinder transport when the polymeric shell is uneven, thus aggregating the colloids. The shell structure was found to depend on the chemical changes that affected organic matter macromolecule conformation and adsorption affinity. In contrast to the above research, others have reported that organic matter inhibits microbe transport due to hydrophobic interactions between microbes and grain surfaces that are coated with organic matter (Bales et al., 1993; Kinoshita et al., 1993). Harvey et al. (2010) recently studied the pH dependency of bacterial attachment to assess the relative effects of the hydrophobic and total fractions of the plume dissolved organic carbon (DOC), anionic and nonionic surfactants, and humic and fulvic acids. Anionic surfactant caused a 11–33% decrease in bacterial attachment at pH 5–6.5, whereas nonionic surfactant caused an increase in attachment likely due to hydrophobic interactions. Humic acid tended to decrease bacteria attachment at pH 5–6.5 likely by altering the surface charge of iron-coated quartz grains and bacteria, whereas the lower molecular weight fulvic acid had much less of an effect on attachment. More recently, it was reported that the concentration of DOC in groundwater can have a large effect upon both the hydrophobicity and zeta potential of bacteria being advected through a granular aquifer (Harvey et al., 2011). Adsorption of pathogens onto mobile colloids can also facilitate their mobility (de Jonge et al., 2004; Jin et al., 2000; Walshe et al., 2010). In summary, the above studies demonstrate that different forms of DOC can affect in different manners the attachment characteristics of pathogens. Consequently, it may be difficult to predict pathogen transport properties in the natural environment when DOC is present.

Due to all of the above complexities, the value of α is frequently considered to be an empirical parameter that is determined from measured breakthrough curves and/or deposition profiles in column experiments. In particular, α is estimated from Equation [3] using fitted values of k_{asw} and correlations for η (e.g., Tufenkji and Elimelech, 2005a). However, even when α is obtained by fitting to the profiles of retained colloids in porous media, substantive deviations from what is predicted from filtration theory have been observed. In particular, retained colloids and/or microorganisms have been observed to become retained at nonexponential deposition rates that produce hyper-exponential (a decreasing rate of deposition coefficient with

distance; Albinger et al., 1994; Baygents et al., 1998; Bolster et al., 2000; Bradford et al., 2003; DeFlaun et al., 1997; Li et al., 2004; Redman et al., 2001; Simoni et al., 1998; Zhang et al., 2001a), nonmonotonic (a peak in retained colloids away from the injection source; Bradford et al., 2006b; Tong et al., 2005), or uniform (Bradford et al., 2009b; Xu et al., 2006) deposition profiles. Deviations between experimental and filtration theory predictions have been attributed to the following reasons: (a) porous media charge variability (Johnson and Elimelech, 1995), (b) heterogeneity in surface charge characteristics of colloids (Bolster et al., 1999; Li et al., 2004; Tong and Johnson, 2007); (c) deposition of colloids in the secondary energy minimum (Hahn et al., 2004; Redman et al., 2004; Tufenkji and Elimelech, 2005a); (d) time dependent attachment (Liu and Johnson, 1995; Tan et al., 1994); (e) colloid detachment (Cortis et al., 2006; Harter et al., 2000; Tufenkji et al., 2003); (f) deposition of colloids in small pore spaces such as those formed at grain-grain contacts (Bradford et al., 2002; Bradford et al., 2003; Bradford et al., 2004; Bradford and Bettahar, 2005; Bradford et al., 2006a; Bradford et al., 2006b; Cushing and Lawler, 1998; Foppen et al., 2005; Li et al., 2004; Tufenkji et al., 2004); (g) soil surface roughness (Kretzschmar et al., 1997; Redman et al., 2001); (h) hydrodynamic drag (Li et al., 2005); (i) aggregation (Bradford et al., 2006b; Chatterjee and Gupta, 2009; Chatterjee et al., 2010); and (j) changes in pore structure (Chen et al., 2009; Mays and Hunt, 2005); and (k) because of the non-granular nature of the porous medium (e.g., Harvey et al., 2008). Further discussion on a selection of these issues is provided in the section below.

Retention processes not only depend on the interaction between the approaching colloid and the collector surface, but are also affected by interactions with attached particles. Approaching particles have been observed to have lower deposition rates as a result of blocking, which refers to the occlusion of the collector surface by already attached particles (Adamczyk et al., 1994; Johnson and Elimelech, 1995). This phenomenon assumes that chemically favorable attachment locations are already filled so incoming particles can only access unfavorable sites and thus fail to be retained. To simulate reductions in the attachment coefficient due to filling of favorable attachment sites, the value of k_{asw} is sometimes multiplied by a blocking function (ψ_{sw}), which is assumed to decrease with increasing colloid mass retention. Random sequential adsorption (Johnson and Elimelech, 1995) and Langmuirian dynamics (Adamczyk et al., 1994) equations have been proposed for ψ_{sw} to describe this blocking phenomenon, with the latter equation given by

$$\psi_{sw} = 1 - \frac{S_{att}}{S_{att}^{\max}} \quad (4)$$

Here S_{att}^{\max} [NM^{-1} ; where N denotes number of particles] is the maximum attached solid-phase colloid concentration. Conversely, approaching particles may experience an increase in deposition rates as a result of ripening,

as attractive colloid-colloid interactions enhance attachment to the collector surface (Chiang and Tien, 1985a, 1985b; Deshpande and Shonnard, 1999; Tien, 1989). As is discussed in later sections, biological activity is suspected to highly influence these two phenomena (Tufenkji, 2007). Enhanced colloid retention during ripening can theoretically be described using a functional form of ψ_{sw} that increases with increasing mass of retained colloids (Chiang and Tien, 1985a, 1985b; Deshpande and Shonnard, 1999; Tien, 1989).

The value of S_{att}^{\max} in Equation [4] can be related to the fraction of the surface area (S_f) of soil that contributes to retention as (Bradford et al., 2009a; Kim et al., 2009a):

$$S_{att}^{\max} = \frac{(1 - \gamma) A_s S_f}{A_c \rho_b} \quad (5)$$

where A_c [L^2N^{-1}] is the cross section area per colloid, A_s [L^2L^{-3}] is the surface area per unit volume of soil, γ [–] is the porosity of a monolayer packing of colloids on the solid surface, and ρ_b is the bulk density of the porous medium. Torkzaban et al. (2007) and Bradford et al. (2011) demonstrate how the value of S_f can be determined from pore-scale water flow simulations and DLVO calculations by conducting a balance of applied hydrodynamic and resisting adhesive torques. Specifically, the cumulative density function of applied hydrodynamic torque is evaluated at a particular resisting adhesive torque to determine S_f (Bradford et al., 2011). According to this approach the value of S_f will be a function of the velocity, grain size and distribution, colloid size, and chemical factors that influence the adhesive interaction (solution composition, and solid and colloid surface chemistries). The influence of chemical heterogeneity may also be accounted for in torque balance calculations to determine an effective value of S_f (Bradford and Torkzaban, 2008).

Agricultural soils and aquifer sediments vary widely in their grain and pore size distributions (Carsel and Parrish, 1988). Calculations presented by Bradford et al. (2006a) demonstrated that a significant fraction of soil pore spaces are likely smaller in size than the diameter of viruses, bacteria, and protozoa of concern. Hence, pathogens may be physically retained in these small pore spaces as a result of straining. The probability of straining to occur depends on the soil texture and has been found to be highest in silty clay and clayey soils and lowest in sandy soils. Conversely, physical exclusion from some of these pore spaces can also produce early breakthrough of pathogens as a result of pore exclusion and associated changes in dispersion (Bradford et al., 2003; Ginn et al., 2002; Ryan and Elimelech, 1996). Experimental observations also support the importance of straining for bacteria and protists (Bradford and Bettahar, 2005; Bradford et al., 2006b; Foppen et al., 2007; Hijnen et al., 2005; Mohanram et al., 2010).

Criteria for straining have traditionally been based on the ratio of the mean particle to the collector grain diameter (d_p/d_g ; Herzig et al., 1970). Geometric calculations of Sakthivadivel (1967) indicated that straining was significant when d_p/d_g was greater than 0.05. Conversely, experimental data suggests that straining may occur when d_p/d_g is as low as 0.002 (Bradford et al., 2002; Bradford et al., 2003; Chen et al., 2008; Li et al., 2004). Pore-scale simulations of colloid trajectories by Johnson et al. (2007d) support the finding of colloid retention at grain-grain contact points when $d_p/d_{50} > 0.005$. The discrepancy between geometric calculations, experimental data, and numerical simulation is likely due to oversimplifications of the complex pore and colloid size and shape distributions in natural systems. For example, bacteria occur in rod, ellipsoidal and spiral shapes (Weiss et al., 1995), and the pore size distribution of soils can span over several orders of magnitude as a result of a wide range of grain sizes and surface roughness. A factor that has received little attention with respect to straining is the shape/aspect ratio of both grains and microorganisms. Xu et al. (2008) suggested that the minor axis of rod shape colloids should be used when investigating straining behavior. Results from Xu and Saiers (2009) indicate that straining can be enhanced in the presence of nonuniform sized suspensions. Tufenkji et al. (2004) found that straining was a strong function of grain angularity and roughness. Li et al. (2006a) and Chen et al. (2008) used microtomography to observe the distribution of deposits relative to pore structure and found that there was a wide distribution of grain and contact morphology even in well-sorted sediments, and that most particle accumulation occurred at grain-grain contacts. Tong and Johnson (2006) found that the effect of angularity of grains was more significant on colloid deposition than grain heterogeneity and surface roughness. A number of studies have examined the effect of surface roughness on the deposition of abiotic and biotic colloids (Darbha et al., 2010; Morales et al., 2009; Shellenberger and Logan, 2002), suggesting that the deposition on a rougher surface is generally greater than on a smooth surface.

Recent research has revealed a complex coupling between hydrodynamics, adhesive interactions, and colloid input concentration on retention. Colloids that weakly interact with the SWI may be translated by hydrodynamic forces to low velocity regions that occur near grain-grain contacts and/or surface roughness (Bradford and Torkzaban, 2008; Bradford et al., 2009b; Bradford et al., 2011; Tufenkji et al., 2004) where they can be immobilized by straining (Bradford and Torkzaban, 2008). Torque balance information indicates that the value of S_f may be very small under these conditions and this has several important implications for pathogen transport. First, advective transport of colloids to low velocity regions can enhance rates of retention and produce hyperexponential retention profiles (Bradford et al., 2009b). Second, small values of S_f imply that transport and retention processes are highly dependent on the colloid pulse input concentration and duration.

Values of input concentration or duration above a threshold can influence the shape of the breakthrough curves and the retention profiles by exhibiting blocking behavior that causes decreased rates of retention over time and uniform amounts of deposition with depth when intraparticle interaction is repulsive (Bradford and Bettahar, 2006; Bradford et al., 2009a). This concentration dependence is expected to be a function of the same factors that influence S_f (velocity, grain size and distribution, pathogen size, and the adhesive interaction). Third, micromodel observations indicated that aggregation of colloids and pathogens can occur at grain-grain contacts and surface roughness locations. This behavior has been associated with nonmonotonic retention profiles (Bradford et al., 2005; Bradford et al., 2006b; Choi et al., 2007; Tong et al., 2008). The above listed trends can help explain observed differences in retention profile shape when straining takes place (Bradford et al., 2006a; Foppen et al., 2007; Xu et al., 2006). Additional research is warranted to examine various types of straining processes, which have been associated with distinct retention profile shapes. More work is also needed on the effects of physical and chemical heterogeneity that lead to preferential deposition and non-first-order deposition rates, including attachment to heterogeneous surfaces, heterogeneous patterns of straining associated with complex pore and grain morphologies, effects of micro-roughness on particle deposition and cellular motion, and potential co-variances in physical and chemical properties.

Much of the experimental and theoretical work done to date concern particle transport in saturated porous media. Less is understood about transport in unsaturated porous media due to the systematic complexities that arise with the presence of an air phase. First, the liquid phase in unsaturated systems is restricted, as both water and air must occupy the void space. Second, capillary forces constrain water flow within regions that have smaller pore spaces. Third, the coexistence of solid, water and air phases gives rise to interfaces that individually or collectively can act as retention sites. All of these factors lead to enhanced pathogen retention with decreasing water content (Chen and Flury, 2005; Crist et al., 2004; Crist et al., 2005; Gargiulo et al., 2007b; Gargiulo et al., 2008; Saiers and Lenhart, 2003; Schäfer et al., 1998b; Torkzaban et al., 2006a; Torkzaban et al., 2006c; Torkzaban et al., 2008a; Wan and Tokunaga, 1997; Wan and Wilson, 1994a; Zevi et al., 2005; Zevi et al., 2009). This behavior has been attributed to increased interfacial sorption of colloids onto the AWI for positively charged or hydrophobic colloids (Cherrey et al., 2003; Saiers and Lenhart, 2003; Schäfer et al., 1998a; Torkzaban et al., 2006c; Wan and Wilson, 1994a, 1994b), physical restrictions imposed by thin water films (film straining; Wan and Tokunaga, 1997; Veerapaneni et al., 2000), retention at the AWS contact line (Chen and Flury, 2005; Crist et al., 2004; Crist et al., 2005; Lazouskaya et al., 2006; Lazouskaya and Jin, 2008; Zevi et al., 2005; Zevi et al., 2009; Zevi et al.,

2012), and immobilization in dead end pores (Gao et al., 2006). In addition, it should be noted that as the water content decreases a greater fraction of the water flows through smaller portions of the pore space (grain-grain contacts, water films, and air-water-solid contact lines) that are associated with straining processes (Gargiulo et al., 2008). Not surprisingly, retention profiles from unsaturated colloid transport studies have been observed to become more hyperexponential as the water content decreased, the sand size decreased, and the colloid size increased (Gargiulo et al., 2007a; Gargiulo et al., 2008; Torkzaban et al., 2008a; Wan and Tokunaga, 1997).

Several studies have investigated the influence of colloid hydrophobicity, dissolution of the entrapped air phase, solution ionic strength, water velocity, and colloid concentration on transport in unsaturated systems. Hydrophobicity of colloids has been observed to have a strong influence on aggregation and associated straining processes in unsaturated porous media (Crist et al., 2005; Gargiulo et al., 2008; Zevi et al., 2005). Sirivithayapakorn and Keller (2003) reported that dissolution of entrapped air also induced the formation of colloid clusters that consequently became retained in pore throats. While various studies have reported that the overall amount of retention in unsaturated porous media increases with IS (Torkzaban et al., 2008a; Zhang et al., 2010b), ionic strength has been observed to have only a marginal influence on colloid retention at the AWI (Lazouskaya et al., 2006; Zevi et al., 2009). An increase in the water flow rate has been generally observed to reduce colloid retention (Torkzaban et al., 2008a). In contrast to results from other saturated systems (Bradford et al., 2009a), Zhang et al. (2010b) found that in both saturated and unsaturated systems, an increase in colloid concentration increased colloid retention and therefore could not be attributed to water saturation effects alone. Rather, the differences in the system pH, surface potential of colloids and sands, and grain shape were ascribed to the observed transport differences between studies. Additional research is needed to better understand the coupled effect of hydrodynamic and adhesive forces, as well as colloid concentration on retention under unsaturated conditions.

The effect of extracellular materials on the cell-surface interactions has been suggested to significantly affect bacterial transport. Experimental approaches to investigate this effect range from the cellular-scale force measurements using atomic force microscopy for *Pseudomonas putida* KT2442, *Burkholderia cepacia* G4 or 866A (Camesano and Logan, 2000; Ginn et al., 2002) to batch adhesion or column transport tests for *E. coli* O157:H7 (Kim et al., 2009a; Kim et al., 2009b; Kim et al., 2010), and *Rhodococcus rhodochrous* (Gargiulo et al., 2007a). An increasing number of studies have found that extracellular macromolecules affect electrophoretic mobility, cell surface hydrophobicity and electrosteric repulsion (e.g., *E. coli* O157:H7,

Cryptosporidium parvum oocysts, and *Rhodococcus rhodochrous*); thus affecting microbial adhesion and transport (Gargiulo et al., 2007a; Kim et al., 2009a; Kim et al., 2009b; Kim et al., 2010; Kuznar and Elimelech, 2004; Liu et al., 2010). Surface macromolecules in protozoa have been recently associated with steric repulsion, as experimental observations reported a decrease in deposition rates under favorable conditions in their presence, and an improvement in filter efficiency post biomolecule denaturing to chemically remove them (Considine et al., 2001; Kuznar and Elimelech, 2005). Reported results indicated that biomolecules can enhance (Burks et al., 2003; Kim et al., 2009b; Rijnaarts et al., 1996; Rijnaarts et al., 1999; Salvucci et al., 2009; Walker et al., 2004), and also hinder transport (Burks et al., 2003; Kim et al., 2010; Rijnaarts et al., 1996; Rijnaarts et al., 1999; Salvucci et al., 2009). Using a proteolytic enzyme to cleave the surface proteins of *E. coli* and *Cryptosporidium parvum* oocysts, past studies have elucidated the role of surface macromolecules in governing the deposition and transport of microbial cells (Kim et al., 2009b; Kim et al., 2010; Kuznar and Elimelech, 2006; Liu et al., 2009; Liu et al., 2010). In particular, surface macromolecules have been reported to invoke electrosteric repulsion for both *E. coli* and *C. parvum* oocysts, which resulted in reduced deposition in otherwise favorable conditions predicted by DLVO theory (Kim et al., 2009a; Kuznar and Elimelech, 2004, 2006; Liu et al., 2009; Liu et al., 2010). Electrosteric repulsion has been implicated with hindered retention of *E. coli* O157:H7 at higher IS and pH conditions (Kim et al., 2009a). Conversely, polymer bridging of surface macromolecules has been found to enhance cell retention and aggregation under low IS conditions (Gargiulo et al., 2007a; Kim et al., 2010). In addition, results of microorganism transport studies have been demonstrated to be sensitive to the method of harvesting and sample handling (Tazehkand et al., 2008), presumably due to conformation changes in the surface macromolecules.

An increasing amount of evidence has been gathered indicating that the degree of intrapopulation variability affects the transport of microorganisms in granular media (Bolster et al., 2000). Further, studies have shown that a single bacterial strain can harbor two subpopulations with differing transport properties (DeFlaun et al., 1997). The existence of two subpopulations that differ markedly in their propensities for attachment to grain surfaces have been termed dual alpha populations (e.g., Bolster et al., 2000). However, it is unlikely that intrapopulation variability in surface characteristics will be limited to only two subpopulations. Fuller et al. (2000) demonstrated that for a single bacterial strain injected into intact cores, there was a range of subpopulations, each possessing a different α value. In addition to its effect upon bacterial surface properties, intrapopulation variability can also affect transport if differences exist in motility behavior. For example, it has been shown that distinct subpopulations within *Pseudomonas aeruginosa* affect the type of motilities (swimming, twitching, and swarming) that are expressed

(Tremblay and Deziel, 2010). It also appears that starvation-induced control of motility differs among subpopulations of the same bacterial species (Wei and Bauer, 1998).

Chemotaxis involves the ability of motile bacteria to change their run lengths by increasing or decreasing their turning frequency in response to changing concentrations of the chemoattractant. Bacterial chemotaxis requires a spatial gradient of an attractant that is steep enough for the bacterium to sense it temporally. Consequently, it can be particularly effective for migrations on the centimeter (column) scale (Barbara and Mitchell, 2003). Measured macroscopic migration rates for motile bacteria moving through a variety of subsurface granular material packed into columns range from 0.4 to 0.5 cm/hr (Barton and Ford, 1995; Harwood et al., 1989; Jenneman et al., 1985; Reynolds et al., 1989). Because the input of nutrients from the surface environment to the subsurface can result in steep vertical gradients (Smith et al., 1991), chemotaxis may be a mechanism by which bacteria can move vertically in groundwater in a direction opposite to that of the mean groundwater flow. Chemotactic migration through granular media depends on the ability of motile bacteria to follow a chemoattractant gradient through a complex network of pores and pore throats. Consequently, run-and-tumble type of motility used by a number of bacterial indicators such as *E. coli*, may be the most effective in translocation through granular media because of their ability to constantly change motility direction (approximately once every second), thus enhancing the likelihood of finding pore-throats leading to the chemoattractant source (Ford and Harvey, 2007).

3.5 Pathogen Release From Animal Waste, Sediment, and Soils

The release of microbial pathogens from land-applied manure and biosolid provides a source of microbes that can be transported in the surface and subsurface. Manure-derived pathogen release greatly depends on conditions favoring detachment of microbial cells from the manure matrix. This detachment is thus dependent on the properties of the matrix material, chemistry of the aqueous phase, and microbial cell properties (e.g., solution pH, ionic strength, organic matter content, cell surface properties) (Ferguson et al., 2003). When manure is exposed to precipitation, pathogen concentrations in runoff water tend to gradually increase to a maximum value and then to decrease over several orders of magnitude to persistently low levels (Bradford and Schijven, 2002; Kress and Gifford, 1984; Mawdsley et al., 1996; Schijven et al., 2004; Tate et al., 2000; Thelin and Gifford, 1983). Pathogen release from manure and soil can occur in water as free microbes or in association with manure and/or soil particles (Dao et al., 2008; Reddy et al., 1981; Tyrrel and Quinton, 2003). Pathogen and indicator microorganism concentrations in runoff water have also been correlated to turbidity (Bradford and Schijven, 2002; Schijven et al., 2004) and phosphohydrolase-labile phosphorus (a

fraction associated with particulate manure; Dao et al., 2008). The pathogen release rates from manure to flowing water have been demonstrated to depend on precipitation rate (Schijven et al., 2004), manure composition and age, land application method (Drapcho and Hubbs, 2003; Hutchison et al., 2004; Schijven et al., 2004; Soupir et al., 2003), and water solution chemistry (Bradford and Schijven, 2002). Pachepsky et al. (2009) observed that the particle size distribution in manure runoff and leachate remained stable after 15 min of runoff initiation. Nonetheless, differences in release rates have been observed for various microbes (Guber et al., 2007a).

Manure contaminated soil and sediment is often implicated as a secondary source of pathogens in flowing water. At the soil surface, pathogen release may occur as a result of raindrop impact and other erosion processes (Boyer, 2008; Park and Mitchell, 1982; Park et al., 1983). These factors are strongly linked to the surface water hydrodynamics (precipitation intensity and durations, water velocity and depth). As such, rates of pathogen release from the soil surface are expected to increase with water velocity (Park and Mitchell, 1982; Park et al., 1983). In brief, microbes will theoretically be released when the applied hydrodynamic torque exceeds the resisting torque due to adhesion, gravity, and/or friction, or when raindrop impact propels particles into solution. Quinton et al. (2001) demonstrated that erosion processes were selective for fine and low-density particles, such as pathogens, compared with larger mineral colloids. Microorganism release from the soil surface is also expected to increase with the number of retained microorganisms, and decrease with the protection afforded to microbes by soil, vegetation, and biological material (Tyrrel and Quinton, 2003).

In the subsurface environment, detachment of pathogens from soils may occur as a result of increased diffusion (Dong et al., 2002; Ryan and Gschwend, 1994; Shen et al., 2007; Simoni et al., 1998), increase in hydrodynamic forces (Bradford et al., 2011; Hubbe, 1984; Sharma et al., 1992; Torkzaban et al., 2007), reduction of adhesive forces (Bergendahl and Grasso, 1999; Lenhart and Saiers, 2003; Ryan and Gschwend, 1994; Torkzaban et al., 2010), or due to transients in water saturation (expansion and/or contraction of water films, and a moving air-water-solid triple point; Cheng and Saiers, 2009; Gao et al., 2006; Lazouskaya and Jin, 2008; Saiers et al., 2003; Saiers and Lenhart, 2003; Shang et al., 2008; Torkzaban et al., 2006b; Zhuang et al., 2007). In addition, cell motility has been reported to influence both attachment and detachment. However, this effect is dependent on the system hydrodynamics and a consensus on the mechanisms behind it is lacking (Becker et al., 2004; McClaine and Ford, 2002).

Colloid detachment is frequently considered to be a diffusion-controlled process. Under saturated conditions, the value of the detachment coefficient, k_{dsu} (T^{-1}), is reported to be a function of the diffusion coefficient and the boundary layer thickness (Ryan and Elimelech, 1996; Ryan and Gschwend,

1994), viz:

$$k_{dsw} = \frac{2D_{\infty}}{\delta_{bl}} = 2v^{0.66} \left(\frac{d_{50}}{2} \right)^{-1.33} \left(\frac{k_B T_K}{6\pi \mu_w r_c} \right)^{0.33} \quad (6)$$

where D_{∞} [L^2T^{-1}] is the colloid diffusion coefficient in bulk solution given by the Stokes-Einstein equation, δ_{bl} [L] is the boundary thickness, μ_w [$ML^{-1}T^{-1}$] is the water viscosity, k_B [JK^{-1}] (J and K denote Joules and Kelvin temperature, respectively) is the Boltzmann constant, and T_K [K] is the absolute temperature. It should be mentioned that k_{dsw} given by Equation [6] does not account for the strength of the adhesive interaction. The analysis associated with Equations [1] and [2] indicates that the value of k_{dsw} may be multiplied by $1-\alpha_1-\alpha_2$ to account for the fraction of colloids that possess sufficient kinetic energy to detach from the SWI at any given time. It is logical to anticipate that a similar expression to Equation [6] could be developed to determine the detachment rate under unsaturated conditions. However, the dependence of δ_{bl} on water saturation is unknown. In addition, the above approach does not account for removal of colloids due to hydrodynamic mechanisms such as rolling, lifting, or sliding (Bergendahl and Grasso, 1998, 1999; Tsai et al., 1991).

A single value of the detachment coefficient is unlikely to account for temporal changes in detachment that will occur as a result of transients in solution chemistry, flow rate, and water content. It is well accepted that changes in solution chemistry (ionic strength, pH, cation exchange, and concentration of organic matter) will impact the electric double layer thickness and/or the zeta potential, thereby altering electrostatic interactions. Chemical perturbations are often used to alter the interaction energy between colloids and surfaces and induce particle attachment (Goldshmid et al., 1973; NocitoGobel and Tobiason, 1996; Shiratori et al., 2007; Tosco et al., 2009) or detachment (Bales et al., 1991; Bradford and Kim, 2010; Grolimund et al., 2001; Lenhart and Saiers, 2003; McDowell-Boyer, 1992; NocitoGobel and Tobiason, 1996; Roy and Dzombak, 1996; Ryan and Gschwend, 1994; Shiratori et al., 2007; Tosco et al., 2009). Ryan and Gschwend (1994) reported that changes in solution chemistry must be great enough to overcome attractive energy interactions in order to successfully achieve detachment. Tosco et al. (2009) reported that chemical perturbations that induced detachment were well correlated with the disappearance of secondary minima wells. Moreover, hysteresis of colloid/microbe retention has been observed with changes in the solution IS (Torkzaban et al., 2010).

Several studies have investigated the effects of sudden changes in moisture content on particle fate during draining and imbibing events (Auset et al., 2005; Cheng and Saiers, 2009; Gao et al., 2006; Saiers et al., 2003; Saiers and Lenhart, 2003; Zhuang et al., 2007; Zhuang et al., 2009). The reported results appear inconsistent, as some studies attribute increased retention to

drainage events (Zhuang et al., 2009), others ascribe increased detachment to drainage (Saiers et al., 2003), and a third set reports increased detachment to imbibition (Auset et al., 2005; Gao et al., 2006; Saiers and Lenhart, 2003). A more recent study indicates that mobilization can occur during both imbibing or draining events because of the pore-scale changes in the air-water configuration (Cheng and Saiers, 2009). The general consensus from the transport studies discussed above is that decreasing the water content of the porous medium (e.g., by drainage or evaporation) will increase colloids retention when steady-state conditions are reached because of increases in the AWI and changes in the flow field. If water films become small enough to cover colloid particles then capillary forces can become dominant and press colloids onto the solid grain's surface with sufficient force to overcome the repulsive energy barrier at the AWI and/or the SWI (Crist et al., 2004). Transients in water content produce a moving AWI that can subsequently mobilize colloids attached to the AWI and/or scour loosely associated colloids from the SWI. An explanation for the reported inconsistencies in colloid release with changes in moisture content is likely due to differences in the interfacial area, the film thickness relative to the colloid size, and the volume of stagnant fluid.

3.6 Pathogen Transport in Agricultural Settings

Figure 3 presents a schematic of the factors and hydrologic pathways that can influence the transport of pathogens in agricultural setting. The initial distribution of pathogens will be highly dependent on the spatial distribution and composition of manure in the field, as well as on locations of contaminated soil, sediment, and water. Some of the pathogens in manure and/or contaminated soil and sediment may be mobilized into water as a result of diffusion, erosion, raindrop impact, and other release mechanisms discussed above. Water at the soil surface can infiltrate or runoff as overland flow, depending on site-specific hydrologic conditions and soil hydraulic properties. Pathogens in infiltrating water may be retained in the vadose zone, migrate downward through the vadose zone and into groundwater, and eventually discharge into surface water bodies. Pathogens also tend to spread out over time by diffusion and dispersion as they propagate. Contaminated surface water and groundwater may eventually be diverted or pumped and used as drinking water or irrigation water. Clearly pathogen transport is strongly influenced by both subsurface and surface hydrologic processes, as complex interactions can potentially take place in surface water, vadoze zone water, and groundwater. It should be mentioned that pathogens may also be transported in bioaerosols, dust, wild animals, insects, farm workers, and machinery (Western-Growers, 2007). However, these routes of dissemination will not be addressed in this review.

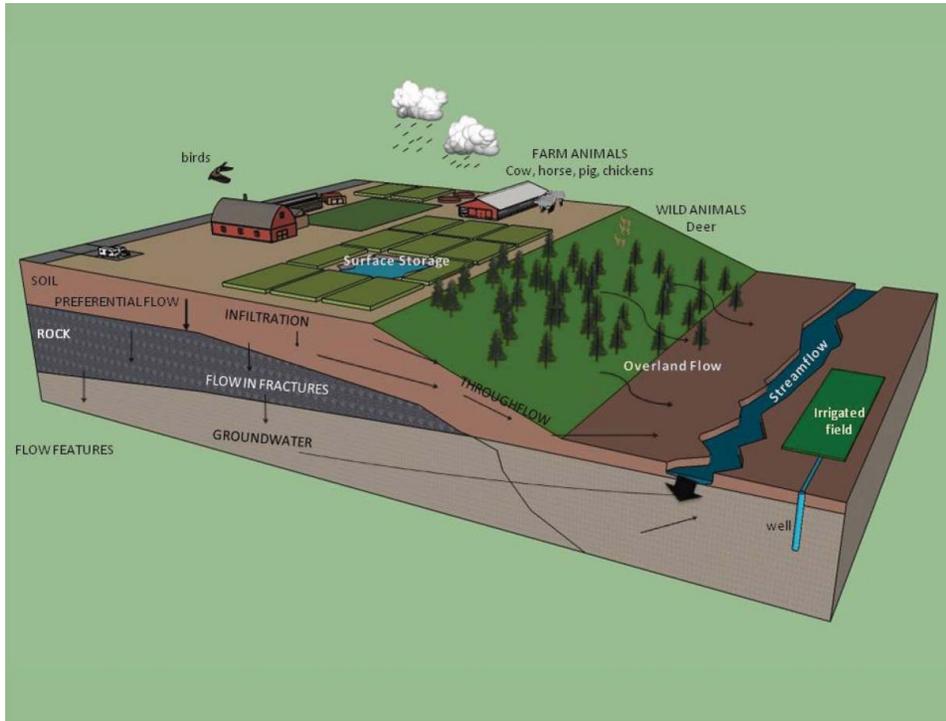


FIGURE 3. A schematic illustrating potential sources and transport pathways for pathogens in agricultural setting (Color figure available online).

Once at the soil surface, pathogens can be rapidly mobilized and exported from soils by overland flow. Overland flow is initiated when the inflow (precipitation, snow melt, or runoff) rate exceeds the soil infiltration rate and fills surface depressions (Hortonian flow), inflow initiates on saturated soil (saturated overland flow), as well as a result of through flow seepage of subsurface water (return flow; Chorley, 1978). The rate and volume of overland flow will tend to increase with inflow, water depth, and soil surface slope; while it will decrease with water infiltration, depression storage, and surface flow resistance from obstructions (Panday and Huyakorn, 2004). Pathogen transport in agricultural settings is especially likely during extreme precipitation events that produce overland flow because raindrop impact and hydrodynamic (shear-induced) erosion can detach and consequently mobilize pathogens. Moreover, the greater velocities by several orders-of-magnitude in overland flow relative to subsurface flow can help translocate mobilized pathogens. Further, overland flow and runoff can produce extremely high pathogen loads to surface water bodies, and have been reported to be the dominant mechanism for pathogen export (Dorner et al., 2006; Jamieson et al., 2004; Krometis et al., 2007; Tyrrel and Quinton, 2003).

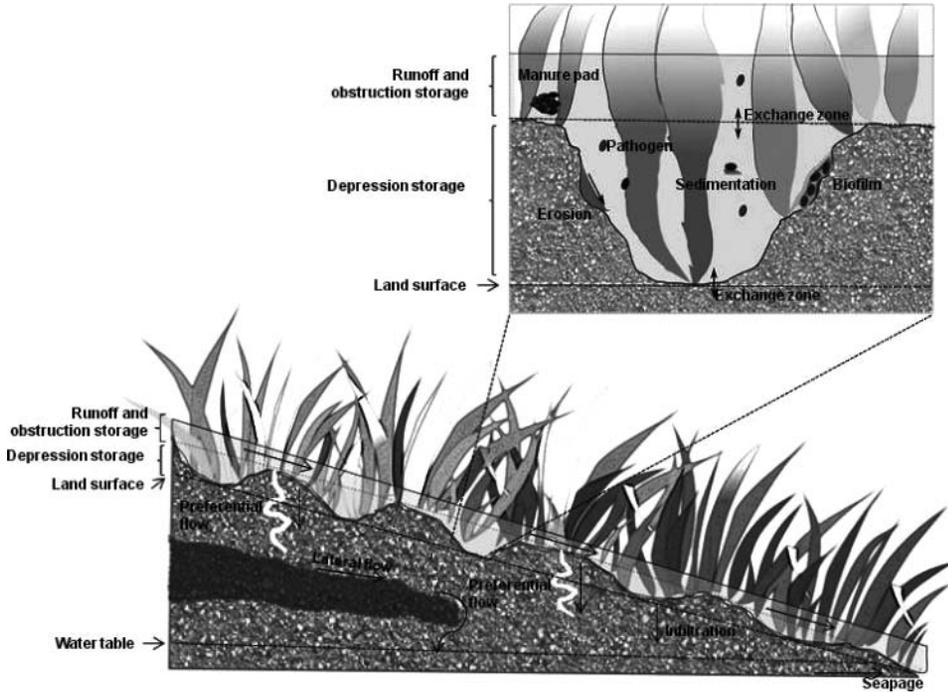


FIGURE 4. A schematic illustrating water flow and pathogen transport in overland flow. Overland flow does not occur until depression storage is filled. Obstructions, preferential flow, soil hydraulic properties and soil layering influence the amount of overland flow and the rate of pathogen transmission. The amount of pathogens in overland flow is highly influenced by exchange with animal waste, soil, depression storage, and vegetation.

Figure 4 presents a conceptual picture of pathogen transport during overland flow and interaction with soil and vegetation. Overland flow may occur as either sheet or rill flow. Sheet flow appears as a thin layer of water with deeper, faster flow near protruding objects. Rill flow is deeper and faster than sheet flow, and it occurs in small channels and gullies. Overland flow can range from laminar to fully turbulent, depending on the water depth, velocity, and raindrop impact (Moore et al., 1990). Patterns of overland flow are complex and vary substantially over time as water first fills surface depressions, which become inter-connected with further water inputs (Darboux and Huang, 2005; Dunne et al., 1991; Thompson et al., 2010). Similarly erosion and flow channeling can yield complex patterns of rill and gully formation over longer timescales (many storms). Depression storage is considered to occur as a result of interaction with surface topography, whereas obstruction storage occurs in protruding vegetation and rocks.

Mobilization of particles, including pathogens, with overland flow is complex. Transport of colloids and pathogens in soils and remobilization

to overland flow have been shown to strongly depend on macropore flow (Harter et al., 2008; Ranville et al., 2005). However, little is known about how macropore flow influences the ensemble probability of pathogen mobilization and ultimate risk of export from agricultural landscapes. Conversely, the presence of vegetation, including installation of vegetated buffer regions or filter strips for runoff management, has been observed to effectively trap and retain a variety of pathogens in overland flow, with reported pathogen removal quantities ranging from 25% to several logs (Atwill et al., 2006; Hussein et al., 2008; Miller et al., 2007; Tate et al., 2006; Trask et al., 2004; Winkworth et al., 2008). Vegetation not only removes pathogens through direct attachment to plant surfaces, but also by increasing flow resistance and thus reducing overland flow velocities, ponding water, and enhancing infiltration (Fiener and Auerswald, 2003). Pathogen removal from overland flow depends strongly on the surface topography, vegetation, and soil texture and structure. Pathogen deposition will likely be enhanced at lower overland flow velocities, lower slopes and water depths, higher surface roughness, and higher vegetation density. However, these effects are debated even for soil erosion (e.g., Darboux and Huang, 2005) and predictive models are not available for pathogen trapping and export at the field scale (Atwill et al., 2002; Edwards, 2003).

Pathogens can readily associate with soil and sediment particles such as clays and organic/inorganic aggregates (Oliver et al., 2007a; Searcy et al., 2005). These pathogen-particle associations can be consequently transported with overland flow (Muirhead et al., 2006a; Soupier et al., 2010). Pathogen removal from overland flow is expected to be enhanced when the microorganisms are associated with soil aggregates, but especially with denser inorganic particles because of increases in sedimentation (Muirhead et al., 2006a; Searcy et al., 2005). However, pathogens released from manure have been observed to often be in a free (unattached) state. Similarly, the presence of manure has been associated with the reduced pathogen association with soil particles (Guber et al., 2007b; Muirhead et al., 2006b; Soupier and Mostaghimi, 2011).

Pathogens in overland flow may also subsequently infiltrate and thus interact with soil water storage, perched groundwater, groundwater recharge, hillslope flow, or agricultural return flow. Hence, pathogen transport will be strongly influenced by repeated interactions with different types of particles and potentially by multiple episodes of surface and subsurface water exchange. Due to the high level of complexity in agricultural system parameters, current understanding about transport and fate of pathogens in agricultural environments remains limited. Gaps in the knowledge remain regarding the distribution of transport pathways available for pathogens to move through; the processes behind pathogen mass exchange between primary sources, land surface, subsurface, and receiving surface water bodies; and the degree to which pathogen association with organic matter and

background particles influences net export of viable pathogens from agricultural landscapes.

Natural geologic processes give rise to heterogeneity of soil, sediment, and rock at every scale. Disturbances by plants, burrowing animals, and anthropogenic alterations to the land further enhance heterogeneity of the shallow subsurface. Subsurface heterogeneity can be characterized in terms of spatially-variable physical properties (e.g., hydraulic conductivity) as well as chemical properties (e.g., mineral composition). Field data show that hydraulic conductivity can vary by orders of magnitude over several centimeters (e.g., Leblanc et al., 1991). One of the great challenges of modern hydrogeology is to determine how much subsurface sampling is needed to adequately assess the physical and chemical spatial variability for a specific process. For transport processes in particular, this variability is known to directly impact flow and therefore pathogen mobility. In particular, soil textural discontinuities are expected to have a large impact on water flow behavior under both saturated and unsaturated conditions by generating complex 3D flow fields. Under saturated conditions, water flow will be enhanced in layers of higher permeability and parallel to lenses; especially when water flow is in the direction of the layer or lens orientation. Water flow under unsaturated conditions is much more complex due to capillary forces that hold water in smaller pore spaces with lower hydraulic conductivity. Consequently, the 3D flow field is saturation dependent, as capillary barriers occur at interfaces of textural discontinuity, causing water to preferentially flow through finer textured soils. Soil layers and lenses tend to form parallel to the soil surface with depth. Hence, there is a significant potential for down slope water flow and pathogen transport at textural interfaces on hillslopes. Discontinuities in soil properties also occurs perpendicular to the soil surface with depth, but are typically less pronounced than variability parallel to the soil surface (Vereecken et al., 2007).

A clear understanding of the role of hydrodynamics, the flow direction, flow focusing, and water content on pathogen retention is needed to accurately characterize and predict pathogen transport in heterogeneous systems at the hillslope scale. However, only limited research efforts have been invested in studying the transport and retention of colloids at textural interfaces and in physically heterogeneous porous media (Bradford et al., 2004; Bradford et al., 2005; Mishurov et al., 2008; Saiers et al., 1994; Silliman, 1995). Colloid transport has been demonstrated to depend on the rates of flow and retention in a variety of materials. Under saturated conditions, finer textured materials have been associated with low permeability that enhances retention. Retention in these porous matrices increases with decreasing flow rate and increasing colloid size. Conversely, Mishurov et al. (2008) found that the maximum colloid recovery did not necessarily occur at greater water content in heterogeneous unsaturated systems. This surprising result counters previously discussed observations of increasing colloid retention with decreasing

water content in homogeneous porous media. In heterogeneous unsaturated systems, greater flow rates and less retention may occur in finer textured soils as a result of capillary barriers that produce higher water contents. However, further research is needed to adequately address these issues.

Preferential flow is a potentially important mechanism for pathogens to bypass portions of the soil matrix. Numerous reviews and original research papers provide detailed discussions of the various processes and conditions leading to preferential flow (Bodvarsson et al., 2003; de Rooij, 2000; Evans et al., 2001; National Research Council, 2001; Ritsema et al., 1993; Simunek et al., 2003; Wang et al., 2004). Preferential water flow can occur as a result of funneling of water at textural interfaces, unstable flow behavior from spatially variable wettability or hysteresis, dynamic capillary properties (nonequilibrium capillary pressure – water content characteristics), macropores, and fractured systems. Each of these factors can potentially accelerate the movement of pathogens through the subsurface at considerably higher velocities than found in the bulk porous matrix. In particular, preferential flow has been widely reported in fractured rock formations and in soils containing wormholes, root channels, and interaggregate fissures (e.g., Beven and Germann, 1982; Vogel et al., 2000). The development of soil structure and aggregates has a large impact on preferential flow and transport processes. Aggregate stability is a function of the clay content and type (Bronick and Lal, 2005; Kjaergaard et al., 2004), sodium adsorption ratio (Ayers and Westcot, 1989), and soil organic matter content (Bronick and Lal, 2005). Upon severe water evaporation periods, a hydrophobic fraction of organic matter can become oriented toward the exterior of aggregates, endowing the soil with a water-repellent coating. The water-repellency nature of these soil aggregates may have a significant effect on pathogen transport.

Many preferential flow processes have been demonstrated to be a function of water content. In particular, larger and more conductive preferential flow channels tend to become active when the rainfall intensity surpasses the infiltration capacity of the soil, leading to ponded infiltration (Cey and Rudolph, 2009; Pot et al., 2005). Colloids and microorganisms can also be transported in preferential flow pathways (Beven and Germann, 1982; Burkhardt et al., 2008; Cey and Rudolph, 2009; Cey et al., 2009; Darnault et al., 2004; Gjettermann et al., 2009; Jarvis, 2007; McDowell-Boyer et al., 1986; McGechan and Lewis, 2002; Nielsen et al., 2010; Passmore et al., 2010; Pang et al., 2008; Powelson et al., 1993). Preferential flow has been implicated in the rapid transport of bacteria to field tile drains (Dean and Foran, 1992; Evans and Owens, 1972; Geohring et al., 1999; Joy et al., 1998; Smith et al., 1972). This literature indicates that the length and continuity of the macropore system and the exchange rate of water between the macropore and the matrix are critical factors in determining the rate of colloid migration in such systems. These are especially important considerations for transport of pathogens because of the potential for greater retention of pathogens in

the matrix as a result of enhanced chemical interactions, lower hydrodynamic forces, and smaller pore spaces. It is also possible that pathogens may be physically excluded from the matrix, and in this case transport of pathogens would exclusively occur in macropores. Optimum conditions for pathogen transport in preferential flow systems will therefore likely depend on the size of the pathogen, matrix pores, and macropores, but little quantitative research has addressed this issue.

Both preferential and overland flow may play important roles in pathogen transport when the soil is near saturated conditions, with the potential of coupling when the two processes occur simultaneously. In particular, rapid preferential flow may decrease the amount and extent of Hortonian type overland flow as illustrated in Figure 4. However, a low permeability soil layer or capillary barrier may also produce rapid lateral flow that can eventually enhance saturated overland and return flow down slope (Germann, 1990). This may partially explain the very rapid (15 m/hr) transport of injected bacteria through hillslope soils (14% slope) overlying fractured saprolite (Rahe et al., 1978). Hence, preferential flow in agricultural settings may need to be examined from a 2D or 3D perspective, rather than its usual simplification as a 1D process. Many challenges remain to accurately identify and parameterize pathogen transport and fate processes during overland flow under the full range of possible transport scenarios, especially when considering the effects of soil structure and heterogeneity over large spatial scales. Additional research is warranted on these topics.

Pathogen survival in aquatic, soil, and sedimentary environments tremendously influences the opportunity for transmission of viable pathogens away from primary sources. Many successful waterborne pathogens have cyst or spore states that protect them from environmental stresses and enable their survival outside of hosts for periods of weeks, months, or even years. Robust opportunistic bacterial pathogens can also potentially replicate in environmental reservoirs. Pathogens that survive for extended periods or maintain viable populations in the environment have a greatly enhanced probability of being subjected to a rapid-transport event such as intense rainfall. These events can mobilize pathogens from manure and soils, and subsequently generate overland flow with much faster rates of pathogen propagation than infiltration and subsurface flow. Similarly, extended survival provides greater opportunity for encountering a high-flow event that will cause rapid and thus long-distance propagation in surface waters.

Little information is available on the spatial and temporal variability in ecosystem-level controls of subsurface microbial transport. However, spatial variability in environmental conditions would be expected for soils and aquifers associated with agricultural lands, because of nonuniform inputs of organic carbon, nitrogen, and other nutrients. For example, in a study by Kinner et al. (2002), a shallow aquifer contaminated with organic

compounds had an abundance of nanoflagellates (protozoa) that fed on bacteria being transported with the contaminant plume. This microbial abundance was directly related to the concentration of groundwater DOC and inversely (and logarithmically) related to the length of time that the dissolved organic compounds had been in the aquifer. Near the source of the organic contamination, it was also found that grazing by protozoa was as important a sink as was deposition of bacteria in the sediment (Kinner et al., 1998). The aforementioned studies suggest that the relative importance of protozoa in the fate and transport of bacterial pathogens and indicators is not spatially uniform in the subsurface, but varies with physical and chemical conditions.

Parasitism of nonindigenous bacteria in the subsurface can occur in the presence of bacteriophage (bacteria-specific viruses) and predatory bacteria such as *Bdellovibrio spp.*, although the spatial distribution of these parasites of bacteria is not well understood. There are a number of phages (coliphages) that are specific for enteric bacterial pathogens and indicator bacteria. For example, *E. coli* acts as a host for many pathogens. Also, although *Bdellovibrio spp.* is known to parasitise a variety of soil bacteria, *B. bacteriovorus* 109J can also parasitise *E. coli* (Rogosky et al., 2006). Some *Bdellovibrio spp.* have been observed to prey on *E. coli* O157:H7 and *Salmonella spp.* (Fratamico and Cooke, 1996).

Ultimately, observed disease transmission in agricultural settings represents the ensemble average of the behavior of highly diverse pathogen populations in highly heterogeneous landscapes, soils, and aquifers, and over highly variable hydrologic and climatic conditions. As many pathogens are produced in copious quantities, but are infectious at low doses, even the delivery of an extremely small fraction of the initial source material can lead to disease outbreaks. Therefore, extended survival of even a small numbers of pathogens can present significant risks of long-distance transport through high-flow pathways or rare rapid-transport events. For some pathogens, like cyst-forming protozoa (e.g., *Cryptosporidium* and *Giardia*), their environmental persistence and high transmissivity afford these organisms high risk potential. Persistent environmentally transmissible phenotypes, occurrence of preferentially transported or preferentially persistent subpopulations, and potential colonization of environmental reservoirs have also been suggested to be important for many other waterborne pathogens.

4. MATHEMATICAL MODELING

4.1 Deterministic Modeling of Pathogen Transport and Fate in Porous Media

Continuum models for pathogen transport and fate in porous media average the heterogeneous water flow field, and microbe transport and fate processes that occur at the pore-scale over a representative elementary volume

(REV) to obtain effective parameters. Due to this averaging process some pore-scale information is lost, and it may not be feasible to include explicit, mechanistic descriptions of all of the individual pore-scale processes that control the transport and fate of microbes in porous media. In this case, pore-scale processes may need to be lumped into effective parameters at the REV scale. Heterogeneity at the REV scale is accounted for in continuum models by deterministically accounting for spatial and temporal distribution of model parameters, and numerically solving the discretized flow and transport equations subject to appropriate initial and boundary conditions.

Microorganism transport and fate models are commonly based on some form of the advection-dispersion equation (Bradford et al., 2003; Corapcioglu and Choi, 1996; Harvey and Garabedian, 1991; Hornberger et al., 1992), in conjunction with Richard's equation for variably-saturated water flow (Richards, 1931). A complete expression of microbe transport that includes exchange to the solid phase and to the air-water interface can be given in one-dimensional form as

$$\frac{\partial \theta_c C}{\partial t} + \rho_b \frac{\partial S}{\partial t} + \frac{\partial A_{aw} \Gamma}{\partial t} = \frac{\partial}{\partial z} \left(\theta_c D \frac{\partial C}{\partial z} \right) - \frac{\partial q_c C}{\partial z} + B_w \quad (7)$$

where C [NL^{-3}] is the microbe concentration in the aqueous phase, S [NM^{-1}] is the microbe concentration retained on the SWI, Γ [NL^{-2}] is the microbe concentration retained on the AWI, θ_c [L^3L^{-3}] is the volumetric water content accessible to microbes, D [L^2T^{-1}] is the hydrodynamic dispersion coefficient for microbes, ρ_b [ML^{-3}] is the bulk density, A_{aw} [L^2L^{-3}] is the air-water interfacial area per unit volume, q_c [LT^{-1}] is the volumetric water flux density for colloids, B_w [$\text{NL}^{-3}\text{T}^{-1}$] represents growth/death of the microbes in the water phase, z [L] is the distance in the vertical direction, and t [T] is the time. The first two terms on the right side of Equation [7] represent the dispersive and advective microbe fluxes, respectively. The second and third terms on the left side of Equation [7] represent the mass-transfer terms from the aqueous phase to/from the SWI and AWI in units of $\text{NL}^{-3}\text{T}^{-1}$, respectively. Due to ion or size exclusion, θ_c may be smaller than the total volumetric water content, θ_w [–]. In this case, the value of q_c may also be different from the total water flux, q_w [LT^{-1}]. Several methods have been proposed to simulate the enhanced velocity of colloids as a result of size exclusion (Bradford et al., 2003; Ginn, 2002; Simunek et al., 2006) and the interested reader is referred to these publications for details on this topic.

The removal of microorganisms from the liquid to the solid phase can be modeled as equilibrium or kinetic processes that are irreversible or reversible. In this work, we assume that exchange of microbes with the SWI and AWI can be divided into equilibrium sorption, attachment, and straining

mechanisms as

$$\rho_b \frac{\partial S}{\partial t} = \rho_b \frac{\partial S_{eq}}{\partial t} + \rho_b \frac{\partial S_{att}}{\partial t} + \rho_b \frac{\partial S_{str}}{\partial t} - B_s \quad (8)$$

$$\frac{\partial A_{aw} \Gamma}{\partial t} = \frac{\partial A_{aw} \Gamma_{eq}}{\partial t} + \frac{\partial A_{aw} \Gamma_{att}}{\partial t} + \frac{\partial A_{aw} \Gamma_{str}}{\partial t} - B_a \quad (9)$$

where subscripts *eq*, *att*, and *str* denote colloid concentrations retained by equilibrium sorption, attachment, and straining retention mechanisms, respectively, B_s [$\text{NL}^{-3}\text{T}^{-1}$] represents growth/death of the microbes on the solid phase, and B_a [$\text{NL}^{-3}\text{T}^{-1}$] represents growth/death of the microbes at the air-water interface.

Equilibrium sorption expressions are commonly used to describe interactions between solid phases and solutes, but are not frequently used to describe microbe retention. A general nonlinear equilibrium expression for microbe sorption that can be incorporated into Equation [8] is given as (Simunek et al., 1998):

$$S_{eq} = \frac{K_d C^\beta}{1 + \xi C^\beta} \quad (10)$$

Where β [–], K_d [$\text{N}^{-\beta+1} \text{L}^{3\beta} \text{M}^{-1}$], and ξ [$\text{N}^{-\beta+1} \text{L}^{3\beta} \text{M}^{-1}$] are empirical coefficients. The Langmuir ($\beta = 1$), Freundlich ($\xi = 0$), and linear sorption ($\beta = 1$ and $\xi = 0$) equations are special cases of Equation [10]. A similar expression to Equation [10] can be used to account for sorption on the AWI by replacing S with Γ . It is crucial to note that equilibrium sorption only delays the transport of suspended cells and does not contribute permanent removal of microorganisms from the liquid phase. However, retarded transport does increase the opportunity for die-off of pathogens through other mechanisms.

Attachment has commonly been assumed to be the dominant mechanism of microbe retention in porous media. Microbe mass-transfer between the aqueous and SWI and AWI as a result of attachment and detachment can be modeled using the following expressions as (Simunek et al., 2006):

$$\rho_b \frac{\partial S_{att}}{\partial t} = \theta_c \psi_{sw} k_{asw} C - \rho_b k_{dsw} S_{att} \quad (11)$$

$$\frac{\partial A_{aw} \Gamma_{att}}{\partial t} = \theta_c \psi_{aw} k_{aaw} C - A_{aw} k_{daw} \Gamma_{att} \quad (12)$$

where k_{aaw} [T^{-1}] is the first-order colloid attachment coefficient to the AWI, k_{daw} [T^{-1}] is the first-order colloid detachment coefficient to the AWI, and ψ_{aw} is a dimensionless colloid retention function on the AWI [–] to account for blocking or ripening (similar to Equation [4]). Only a fraction of the SWI (f_{sw}) and AWI (f_{aw}) may be accessible for attachment due to (a) size exclusion of

mobile colloids from small pore spaces or thin water films (Bradford et al., 2003; Bradford et al., 2006a; Wan and Tokunaga, 1997); and (b) applied hydrodynamic torques that are greater than the resisting adhesive torque (Bradford and Torkzaban, 2008; Bradford et al., 2011; Torkzaban et al., 2007). Hence, the first term on the right side of Equations [11] and [12] may need to be multiplied by f_{sw} and f_{aw} , respectively. The fraction of the solid surface area that is available for attachment can be estimated from expressions given in Simunek et al. (2006) or using the torque balance approach outlined in Bradford and Torkzaban (2008).

Microorganism transport and fate has also been modeled using two kinetic attachment/detachment sites to account for porous media charge variability (Schijven and Simunek, 2002). The one- and two-site kinetic attachment models are mathematically equivalent when only a single value of detachment is considered. However, differences in the model formulations occur when separate detachment rates or blocking terms are considered on each kinetic site. Some researchers have attempted to separate the influence of attachment and straining by using separate kinetic sites for each retention mechanism. Bradford et al. (2003) modeled attachment and detachment using a formulation similar to Equation [11] and a simple irreversible first-order kinetic expression for straining as

$$\rho_b \frac{\partial S_{str}}{\partial t} = \theta \psi_{str} k_{str} C \quad (13)$$

where k_{str} [T^{-1}] is the first-order straining coefficient and ψ_{str} [–] is a dimensionless colloid straining function. A flexible, empirical, form for ψ_{str} was used to account for time and depth dependent straining processes as (Bradford et al., 2005):

$$\psi_{str} = H(z - z_o) \left(1 - \frac{S_{str}}{S_{str}^{max}} \right) \left(\frac{d_{50} + z - z_o}{d_{50}} \right)^{-\Omega} \quad (14)$$

where $H(z - z_o)$ is the Heaviside function, z_o [L] denotes the depth of the column inlet or the textural interface, S_{str}^{max} [NM^{-1}] is the maximum solid phase concentration of strained colloids, and Ω [–] is an empirical parameter that controls the shape of the (power law) spatial distribution. The second term on the right hand side of Equation [14] accounts for filling and accessibility of straining sites in a manner similar to the Langmuirian blocking approach (Adamczyk et al., 1994). The third term (Bradford et al., 2003) assumes that colloid retention by straining occurs primarily at the column inlet or textural interface, because the flow field is not yet fully established and colloids therefore have increased accessibility (an advective flux) to small pore spaces. The apparent number of small dead-end pores was hypothesized to decrease with increasing distance since size exclusion and/or limited

advection and transverse dispersivity tend to keep mobile colloids within the larger networks, thus bypassing smaller pores.

Xu et al. (2006) proposed an alternative formulation to simulate straining behavior in saturated porous media as

$$\frac{\rho_b}{n} \frac{\partial S_{str}}{\partial t} = k_{str} C^{-\frac{S_{str}}{\lambda}} \quad (15)$$

where n [–] is porosity, λ [NM⁻¹] is the straining capacity coefficient. In this case, the value of λ quantifies an exponential decline in the straining rate as S_{str} increases and the straining locations fill. These authors reported that straining needed to be considered when d_p/d_{50} was greater than 0.008, and that the value of λ increased in a linear fashion with increasing d_p/d_{50} .

In comparison to colloid attachment, relatively little research has addressed straining processes in porous media and many of these processes are still not fully understood. It is interesting to note that Equations [14] and [15] predict very different shapes in the colloid retention profiles. Equation [14] initially predicts a hyper-exponential profile when $\Omega > 0$. Conversely, Equation [15] predicts a profile that is less than exponential in shape, similar to a Langmuirian blocking function. Direct comparison of the values of k_{str} obtained from these two models of straining is therefore not feasible due to the differences in the predicted profile shape (e.g., the depth dependency in k_{str}). Results from Bradford and Bettahar (2006) and Bradford et al. (2007), however, suggest that straining will be a strong function of system hydrodynamics, solution chemistry, and colloid concentration. All of these factors have been reported to influence the shape and/or magnitude of the colloid deposition profiles and this may partially explain differences in the predicted model behavior.

Some researchers have lumped unsaturated retention processes into attachment and straining sites due to the difficulty in separately characterizing all of these retention processes (Gargiulo et al., 2007a; Gargiulo et al., 2008; Torkzaban et al., 2008a). Alternatively, Wan and Tokunaga (1997) accounted for film straining in unsaturated porous media using the following kinetic expression:

$$\frac{\partial (A_{aw} \Gamma_{str})}{\partial t} = \theta_c \omega_{str} C \quad (16)$$

with

$$\omega_{str} = \omega_i P \left(\frac{d_m}{d_f} \right)^\zeta \quad (17)$$

where ω_{str} [T⁻¹] is the film straining rate coefficient, P [–] is the probability of pendular ring discontinuity, d_m [L] is the microbe diameter, d_f [L] is the thickness of the water films, and ζ [–] and ω_i [T⁻¹] are empirical parameters.

The value of P was approximated using an integral normal probability function that depends on the matrix pressure and a critical matrix pressure when the film disconnects. The value of d_f was determined using the Langmuir film thickness equation (Langmuir, 1938) but other expressions may be used as well (Tuller et al., 1999).

Incorporation of biological factors that influence the fate and transport of microorganisms into predictive mathematical models remains a challenge. Specific factors that influence pathogen survival and growth were discussed earlier. The net rate of pathogen growth, death, and inactivation in the water, solid, and air phases may be modeled as follows:

$$B_w = \theta_w (\Lambda_w - X_w) C \quad (18)$$

$$B_s = \rho_b (\Lambda_s - X_s) S \quad (19)$$

$$B_a = A_{aw} (\Lambda_a - X_a) \Gamma \quad (20)$$

where X [T^{-1}] is a death/inactivation coefficient, Λ [$NL^{-3}T^{-1}$] is the microbe growth coefficient, and the subscripts w , s , a denote the water, solid, and air phases, respectively. Equations [18–20] model the net growth, inactivation, die-off, or survival of bacteria, virus, and protozoa using first-order kinetics (Charles et al., 2008; Crane and Moore, 1986; Himathongkham et al., 1999; Lang and Smith, 2007; Pachepsky et al., 2006). Below we specifically address modeling of X_w and Λ_w , although similar expressions may be developed for growth and death/inactivation on the solid and air phases.

Temperature has commonly been regarded as one of the most critical factors influencing microorganism survival in porous media. The decline of viruses during transport through the subsurface has often been modeled assuming a temperature-dependent, first-order inactivation coefficient with time (Vilker and Burge, 1980). The effects of temperature on survival may be described using variations of the Arrhenius equation (Stumm et al., 1981):

$$X_w(T_k) = X_r \exp \left[\frac{E_a(T_k - T_r)}{RT_k T_r} \right] \quad (21)$$

where T_k is temperature in K, R ($8.3144621 \text{ J mol}^{-1} \text{ K}^{-1}$) is the universal gas constant, E_a (J mol^{-1}) is the activation energy, X_r [T^{-1}] is the reference inactivation/death rate at a reference temperature (T_r , K). In order to solve Equation [21] knowledge of the temperature at a given location and time is required. Heat movement in porous media can be modeled using an advection-dispersion type equation (e.g., Simunek et al., 2008).

In addition to temperature, first-order death/inactivation coefficients have also been reported to depend on a wide variety of abiotic (soil type,

pH, presence of toxic substances, dissolved oxygen, salinity, moisture content, and nutrient availability) and biotic (initial microbial community and population density, competition, predation, and biofilms) factors that were discussed earlier. Only limited attempts have been made to empirically correlate inactivation rate coefficients to these factors (Cerf et al., 1996; Reddy et al., 1981). Considering the complexities involved, at this point it appears a difficult task to predict the inactivation rate from the constituent properties of the microorganisms and physical factors. It is foreseeable that inactivation coefficients will have to be considered as site-specific parameters that can be measured or calibrated by fitting.

Experimental observations presented earlier indicate that microbial survival frequently does not follow a constant inactivation rate (Charles et al., 2008; John and Rose, 2005; Petterson et al., 2001). Some have proposed that viruses in the environment are present in subpopulations that can become inactivated in sequences of different rates (Chrysikopoulos and Vogler, 2004; Molin and Cvetkovic, 2010). The mathematical complexities of representing sequential inactivation by a set of discrete first-order rate coefficients, each governing a different inactivation phase, have been managed by approximating sequential inactivation with a time dependent pseudo first-order rate coefficient (Peleg and Cole, 1998; Sim and Chrysikopoulos, 1996). For example, the Weibull model (e.g., Peleg and Cole, 1998) assumes that the inactivation coefficient may be described as

$$X_w(t) = mb^{-m}t^{m-1} \quad (22)$$

where b [T] and m [–] are empirical parameters. Fernandez et al. (2002) used the Weibull model to simulate the temperature and pH dependent survival of *Bacillus cereus* by making b a function of these variables. There are many other inactivation models, including a log-exponential model (Oliver et al., 2006; Oliver et al., 2010) and those summarized by Crane and Moore (1986). The choice of the model should balance the mathematical complexity and the accurate representation of the relevant processes so that a better prediction of microbial survival can be made to foster management and/or policy decisions based on sound science.

Unlike bacteria, pathogenic viruses and protozoa are obligate intracellular parasites, and thus remain biochemically inert in an extracellular environment. Growth kinetics of pathogenic viruses and protozoa in the environment can therefore be neglected. In contrast, it is now recognized that pathogenic bacteria may sometimes grow in the environment depending on the conditions and this poses a serious concern (Berg et al., 2005; Oliver et al., 2010; Pepper et al., 2006; Reddy et al., 1981; Zaleski et al., 2005b). The logistic model (Verhulst, 1838) combines two ecological processes: growth and competition. It assumes that survival is a function of the pathogen concentration and gives the growth/survival coefficient

as

$$\Lambda_w(C) = \Lambda_o(1 - C/C_{max}) \quad (23)$$

where Λ_o [T^{-1}] is the growth rate in the absence of competition, and C_{max} [NL^{-3}] is the maximum concentration that is associated with the upper limit of population growth. The population increases when $C < C_{max}$, reaches a plateau at $C = C_{max}$, and decreases when $C > C_{max}$. Semenov et al. (2009) found that the logistic model provided a superior description of survival of *E. coli* O157:H7 and *Salmonella enterica* serovar Typhimurium in cattle manure and slurry amended soils than linear, exponential, or Weibull survival models.

When only growth of bacterial populations is modeled, then Monod, Tessier, Contois, or Andrews expressions or their extended versions are typically employed. These models describe the correlation between substrate concentration and specific growth rates with the assumption that saturation kinetics for the cellular population can be reached. Classic Monod kinetics (Monod, 1942) relates the growth rate of a particular microbial population to the concentration of a limiting nutrient (N_g , ML^{-3}) in the environment as

$$\Lambda_w = \frac{\Lambda_{max}N_g}{K_N + N_g} \quad (24)$$

where Λ_{max} [T^{-1}] is the maximum specific growth rate when $N_g \gg K_N$ and K_N [ML^{-3}] is the half-maximum concentration. A modified Monod expression to account for utilization of electron donors, *ed*, and electron acceptors, *ea*, as presented by Mohamed et al. (2007) and Bailey and Ollis (1977) is written as

$$\Lambda_w = \Lambda_{max} \left(\frac{A_{ea}}{K_{ea} + A_{ea}} \right) \left(\frac{A_{ed}}{K_{ed} + A_{ed}} \right) \quad (25)$$

Here, K_{ea} [ML^{-3}] is the half saturation coefficient for the electron acceptor of the microbial species, K_{ed} [ML^{-3}] is the half saturation coefficient for the electron donor of the microbial species, A_{ed} [ML^{-3}] is the aqueous phase concentration for electron donor, and A_{ea} [ML^{-3}] is the aqueous phase concentration of electron acceptor. Expressions similar to Equation [25] have been developed to account for the use of multiple substrates, yield coefficients, and inhibition functions (Rathfelder et al., 2000).

Tessier (1942) uses an alternative expression to model growth rate as a function of the saturation constant (Y , ML^{-3}), by:

$$\Lambda_w = \Lambda_{max}(1 - \exp^{-N_g/Y}) \quad (26)$$

Contois (1959) presented a model that sets the specific growth rate as a function of the population density and the concentration of the limiting

nutrient. Contois kinetics best describes the macroscopic behavior of biomass as a function of the ratio of substrate to biomass. Moreover, the specific growth rate from this model restricts the kinetic growth process to the available surface area, which limits the mass-transfer of substrate to the biomass, viz.

$$\Lambda_w = \Lambda_{\max} \left(\frac{N_g/H}{K_N + N_g/H} \right) \quad (27)$$

where H [ML^{-3}] is the biomass concentration.

Andrews (1968) presented a model that predicts the specific growth rate as inhibited by substrate or product constituents as

$$\Lambda_w = \frac{\Lambda_{\max} N_g}{K_N + N_g + N_g^2/K_i} \quad (28)$$

where K_i [ML^{-3}] is the substrate inhibition constant.

Several limitations to the above listed growth kinetic models make predictions of the dynamic behavior of soil ecosystems subjected to environmental perturbations a difficult task. The first limitation to the above models is that Λ_{\max} must be determined independently from saturation constants, which is commonly measured from batch cultures. Second, additional equations must be developed to account for the limiting nutrient or substrate in the growth equation. Third, conditions that cultures experience in batch test are assumed to be both homogeneous and at steady-state, and may not translate well to complex, dynamic soil and aquifer environments. Fourth, responses to transient conditions (even if they are well defined) are difficult to capture as they are usually delayed. Fifth, multiple correlated parameters may give similar fits to the measured data, resulting in the problem of parameter identifiability (Beck and Arnold, 1977; Liu and Zachara, 2001).

Chemotactic migration toward or away from chemical gradients has been integrated into bacterial transport models (Nelson and Ginn, 2001) by including cellular dynamics simulations with CFT particle trajectory analysis. However, because accurate modeling of the role of chemotaxis requires a priori knowledge of the gradients of the chemoattractant or chemorepellant and the appropriate chemotactic parameters for the bacterium of interest, the incorporation of chemotaxis in predictive subsurface bacterial transport models would be difficult.

4.2 Alternative Model Formulations

Under unfavorable attachment conditions the profiles of retained particles in porous media have been reported to exhibit significant deviations from the exponential shape that is predicted by filtration theory. In an attempt to account for these discrepancies a variety of two-site chemical nonequilibrium

(Bradford et al., 2003; Schijven and Simunek, 2002), physical nonequilibrium (Cherrey et al., 2003), chemical and physical nonequilibrium (Leij and Bradford, 2009), multispecies models (Bradford et al., 2006b; Chatterjee and Gupta, 2009), dual permeability (Bradford et al., 2009b; Yuan and Shapiro, 2011), and stochastic models (Bolster et al., 1999; Bradford and Toride, 2007; Li et al., 2004; Shapiro and Bedrikovetsky, 2010; Tufenkji et al., 2003; Tufenkji and Elimelech, 2005b) have been developed. It should also be mentioned that alternative forms of the transport equation (Equation [7]) have been developed to account for observed deviations in dispersion and pathogen detachment using the fractional advection dispersion equation (e.g., Benson et al., 2000) and the continuous time random walk approach (Cortis et al., 2006), respectively. Several representative model formulations to account for some of these limitations are briefly discussed subsequently.

TWO-REGION MODELS

Dual-permeability models have commonly been used to study preferential and nonequilibrium flow and solute transport in structured soils and fractured rocks (Gerke, 2006; Simunek et al., 2003; Simunek and van Genuchten, 2008). In this case, the dual-permeability model partitions the pore space into two regions that have fast (fracture) and slow (matrix) rates of advective and dispersive transport of solutes. More recently dual-permeability models have also been used to simulate different colloid retention mechanisms that occur in fast (region 1) and slow (region 2) velocity regions of homogeneous porous media (Bradford et al., 2009b; Yuan and Shapiro, 2011). This approach is somewhat analogous to multiphase flow and transport models that partition the pore space into regions accessible for the wetting (small pore spaces) and nonwetting (large pore spaces) phases. The small pore spaces in the dual-permeability model are assumed to maintain continuity by slow flow adjacent to the solid phase, in crevice sites near grain-grain contacts, and in small pores in the same way as the wetting phase in multiphase systems.

The governing equations for water flow in the dual-permeability model are well known and are available in the literature (Gerke and van Genuchten, 1993a, 1993b; Simunek and van Genuchten, 2008). The corresponding 1-dimensional dual-permeability equations for local scale colloid transport and retention are as follows:

$$\frac{\partial (\theta_{w1}C_1)}{\partial t} = -\frac{\partial J_1}{\partial z} + \frac{\Gamma_s}{1-w} - \theta_{w1}k_1C_1 + \rho_{b1}k_{\text{det}1}S_1 \quad (29)$$

$$\frac{\partial (\theta_{w2}C_2)}{\partial t} = -\frac{\partial J_2}{\partial z} - \frac{\Gamma_s}{w} - \theta_{w2}k_2C_2 + \rho_{b2}k_{\text{det}2}S_2 \quad (30)$$

$$\frac{\partial (\rho_{b1}S_1)}{\partial t} = \theta_{w1}k_1C_1 - \rho_{b1}k_{\text{det}1}S_1 - \frac{\rho_{b1}k_{12}S_1}{1-w} \quad (31)$$

$$\frac{\partial (\rho_{b2} S_2)}{\partial t} = \theta_{w2} k_2 C_2 - \rho_{b2} k_{det2} S_2 + \frac{\rho_{b1} k_{12} S_1}{w} \quad (32)$$

$$\Gamma_s = \omega_{12} (1 - w) \theta_{w1} (C_2 - C_1) \quad (33)$$

where C_1 and C_2 [NL^{-3}] are the liquid phase concentrations of colloids in regions 1 and 2, S_1 and S_2 [NM^{-1}] are the solid phase concentrations of colloids in regions 1 and 2, θ_{w1} and θ_{w2} are the volumetric water contents in regions 1 and 2 [-], ρ_{b1} and ρ_{b2} are the bulk densities in regions 1 and 2 [ML^{-3}], k_1 and k_2 [T^{-1}] are the first order colloid retention rate coefficients in regions 1 and 2, k_{det1} and k_{det2} [T^{-1}] are the first-order detachment coefficients in regions 1 and 2, J_1 and J_2 [$\text{NL}^{-2}\text{T}^{-1}$] are the total solute fluxes (sum of the advective and dispersive flux) for colloids in regions 1 and 2, w_{12} [T^{-1}] is a coefficient for colloid exchange between liquids in regions 1 to 2, k_{12} [T^{-1}] is a coefficient for transfer of colloids from solid phase region 1 to 2, and w is the ratio of the volume of region 2 to the total volume (volume of region 1 + volume of region 2). The term Γ_s [$\text{NL}^{-3}\text{T}^{-1}$] accounts for aqueous phase mass exchange of colloids between regions 1 and 2. Equations [29–33] were written in terms of local scale mass balances of regions 1 and 2. To formulate the equations in terms of the total pore space, the mass balance equations for regions 1 and 2 need to be multiplied by $(1-w)$ and w , respectively.

The dual permeability model formulation is quite flexible and has frequently been used to describe transport processes in structured soils (Simunek and van Genuchten, 2008), as well as hyperexponential (Bradford et al., 2009b) and nonmonotonic (Yuan and Shapiro, 2011) retention profiles in homogeneous porous media. Bradford et al. (2009b) demonstrated that low amounts of advective transport to low velocity regions of the pore space produced hyperexponential retention profiles. Yuan and Shapiro (2011) reported that solid phase migration of colloids was a sufficient condition to produce nonmonotonic retention profiles. These simulation results suggest that the dual-permeability model provides a promising physical interpretation for pathogen retention under unfavorable attachment conditions. Unfortunately, the dual permeability model parameters are still largely empirical and obtained by optimization to experimental data. To date, no model exists that provides reliable predictions of pathogen transport and retention under unfavorable conditions, even under relatively simple, well-defined conditions.

It should be mentioned that the dual permeability model can be simplified to obtain a variety of other two region models. For example, the dual permeability model is equivalent to the physical and chemical nonequilibrium (PCNE) model when $J_2 = 0$. An analytic solution for the PCNE model was obtained by Leij and Bradford (2009). These authors developed expressions for the first three time moments of the solutions to elucidate the impact

of transport parameters on the mean, variance, and skewness of breakthrough curves. The physical nonequilibrium model (e.g., van Genuchten and Wierenga, 1976) can be obtained from the dual permeability model when $J_2 = 0$, $k_1 = 0$, and $k_2 = 0$.

PROBABILISTIC MODELS

The deterministic modeling approaches discussed above use a specified set of input parameters to determine a unique set of model outputs (i.e., the aqueous and solid phase pathogen concentrations at a given location and time). In probabilistic modeling approaches input parameters such as pathogen retention, pathogen survival, and the water velocity may be described using probability density functions. A variety of probability density functions have been developed for this purpose. A commonly used log-normal probability density function, $F(p_1)$, for an arbitrary parameter p_1 is defined as

$$F(p_1) = \frac{1}{p_1 \sigma_1 \sqrt{2\pi}} \exp \left[-\frac{Y_1^2}{2} \right] \quad (34)$$

where σ_1 is the standard deviation of the log-normal probability density function, and Y_1 is the normalized log-transformed variable defined as

$$Y_1 = -\frac{\ln(p_1) - \mu_1}{\sigma_1} \quad (35)$$

Here $\mu_1 = \ln(\langle p_1 \rangle) - 0.5\sigma_1^2$ is the mean value of the log-normal probability density function, and $\langle p_1 \rangle$ is the ensemble average of p_1 . The subscript 1 is used on σ_1 , Y_1 , and μ_1 to identify parameters associated with the stochastic parameter 1. Model parameters may also be easily described using other probability density functions. For example, colloid retention coefficients have been described using normal, log-normal, and various bimodal distributions (Bradford and Toride, 2007; Tufenkji et al., 2003; Tufenkji and Elimelech, 2005b). Microorganism retention and survival may also be described using similar probability density functions to reflect differences of subpopulations (Charles et al., 2008; Coroller et al., 2006; Crane and Moore, 1986; Petterson et al., 2001).

When model parameters are correlated, then joint probability density functions are defined. For example, when two stochastic parameters, p_1 and p_2 , are log-normally distributed the joint probability density function is defined as

$$F(p_1, p_2) = \frac{1}{2\pi \sigma_1 \sigma_2 p_1 p_2 \sqrt{1 - \rho_{12}^2}} \exp \left[-\frac{Y_2^2 - 2\rho_{12} Y_1 Y_2 + Y_1^2}{2(1 - \rho_{12}^2)} \right] \quad (36)$$

Here subscript 2 is used on σ_2 , Y_2 , and μ_2 to identify parameters associated with the stochastic parameter 2. The parameter ρ_{12} is the correlation coefficient between Y_1 and Y_2 and is defined as

$$\rho_{12} = \langle Y_1 Y_2 \rangle = \int_0^\infty \int_0^\infty Y_1 Y_2 F(p_1, p_2) dp_1 dp_2 \quad (37)$$

When Y_1 and Y_2 are perfectly correlated then $\rho_{12} = 1$, when they are uncorrelated $\rho_{12} = 0$, and when they are perfectly inversely correlated $\rho_{12} = -1$.

In the Monte Carlo approach (e.g., Rubinstein and Kroese, 2008) model inputs are randomly generated from selected probability density functions, and then iteratively used in a deterministic model such as those described in the preceding sections. The model outputs can subsequently be analyzed statistically to determine probability distributions, mean values, and confidence intervals. Some probabilistic modeling approaches use analytic solutions to simplify transport problems and determine the mean aqueous and solid phase pathogen concentrations at specific locations and times by integrating the analytic solution over the parameter distribution (Bradford and Toride, 2007; Tufenkji and Elimelech, 2005b). For example, the mean aqueous and solid phase pathogen concentrations for two joint log-normally distributed parameters p_1 and p_2 are given as

$$\langle C(z, t) \rangle = \int_0^\infty \int_0^\infty C(z, t; p_1, p_2) F(p_1, p_2) dp_1 dp_2 \quad (38)$$

and

$$\langle S(z, t) \rangle = \int_0^\infty \int_0^\infty S(z, t; p_1, p_2) F(p_1, p_2) dp_1 dp_2 \quad (39)$$

where $C(z, t; p_1, p_2)$ and $S(z, t; p_1, p_2)$ are the aqueous and solid phase colloid concentrations determined from the analytical solution. The variance in aqueous and solid phase pathogen concentrations is given as $\langle C(z, t)C(z, t) \rangle - \langle C(z, t) \rangle^2$ and $\langle S(z, t)S(z, t) \rangle - \langle S(z, t) \rangle^2$, respectively. It should be mentioned that if one of the stochastic variables is v then the flux concentration is defined as $\langle v C(z, t) \rangle / \langle v \rangle$. This same approach may be applied to an arbitrary number of stochastic parameters by integrating the product of the analytic solution and independent or joint probability density functions with respect to the stochastic parameters.

Probabilistic models have been used to improve the description of microbe and colloid breakthrough curves and retention profiles in column-scale studies (Bolster et al., 1999; Bradford and Toride, 2007; Chatterjee and Gupta, 2009; Cortis et al., 2006; Li et al., 2004; Tufenkji et al., 2003; Tufenkji

and Elimelech, 2004b, 2005a, 2005b). In these works various stochastic parameters, probability density functions, transport models, and the solution techniques were considered. Most of these probabilistic models have considered stochastic distributions of the attachment coefficient to account for nonexponential retention profiles that were attributed to chemical heterogeneity of the microbe/colloids and grain surfaces. The hypothesis of charge variability has been invoked for a variety of colloids, including microorganisms (Simoni et al., 1998) and latex microspheres (Li et al., 2004; Tong and Johnson, 2007; Tufenkji and Elimelech, 2005b). Variations in surface charge and surface properties of microorganisms can occur as a result of differences in growth stage, metabolic activity, and genetic differences (Bolster et al., 2009). Charge heterogeneity on polystyrene latex particles has been measured and attributed to separation of ion-rich and ion-poor components of the polymer on the particle surface (Tan et al., 2005). Heterogeneities may arise on solid surfaces as a result of roughness (Hoek and Agarwal, 2006), and chemical impurities such as absorbed ions, metal oxides, organics, clays, and hydroxyl groups on silica surfaces (Song et al., 1994; Tufenkji and Elimelech, 2005a; Vaidyanathan and Tien, 1991). However, Johnson and Li (2005) demonstrated that porous media charge variability and/or the influence of the DLVO secondary energy minima should theoretically be consistent with an exponential deposition profile.

If the colloids are not completely monodispersed, then the distribution of colloid sizes is expected to produce a related distribution of retention coefficients. Chatterjee and Gupta (2009) developed a probabilistic model to account for the colloid size distribution and demonstrated that it could produce hyperexponential retention profiles. This model assumes aggregation does not occur during transport, and that the distribution of attachment coefficients can be determined by the initial colloid size distribution. Conversely, Bradford et al. (2006b) developed a two-species model for monodispersed and aggregated *E. coli* O157:H7 cells that accounted for different rates of transport and retention for each species, and aggregation during transport. This model formulation was able to reproduce observed nonmonotonic retention profiles. Cortis et al. (2006) used a continuous time random walk formulation to evaluate the long-term release often observed with *C. parvum* in porous media (e.g., Harter et al., 2000), and showed that this can be attributed to ongoing attachment and detachment associated with variability in the oocyst population and/or grain-scale heterogeneity in the porous medium.

Probabilistic models have also been developed to account for variability in the velocity distribution on colloid retention (Bradford and Toride, 2007). The stochastic stream tube model represents the complex three-dimensional flow field in porous media by a bundle of one-dimensional stream tubes that do not interact (no mixing). Simulation results indicate that variations

in the attachment coefficient or the average pore water velocity can both produce hyperexponential retention profiles. In addition, the shape of the deposition profile was found to be very sensitive to the correlation between stochastic parameters. When the attachment and detachment coefficients were stochastic and positively correlated, then nonmonotonic retention profiles were obtained. Conversely, when pore water velocity and attachment coefficient were stochastic and inversely correlated, then hyperexponential retention profiles were obtained.

An alternative approach for representing geologic heterogeneity in mathematical models is to sample a portion of the subsurface and to assign properties to the remainder of the domain of interest using a mathematical model to generate a relevant heterogeneity pattern. Anderson (1997) provided a review of commonly used stochastic continuum methods for representing aquifer heterogeneity. These approaches have also been successfully used in fractured rock systems (e.g., Day-Lewis et al., 2000). The simplest form involves generating a Gaussian random field, where the heterogeneity structure is continuous and spatially periodic (see Deutsch and Journel, 1997; Tompson et al., 1989). It has been shown that at least portions of some aquifer hydraulic conductivity heterogeneity may be modeled by this type of geologic structure (for granular media, Boggs et al., 1992; Leblanc et al., 1991; Sudicky, 1986; for fractured rock, Neuman and Depner, 1988).

The spatially continuous model of heterogeneity breaks down for situations where geologic structure changes such that the hydraulic conductivity pattern is affected. There has been much progress in derivation and utilization of geologic models to more accurately account for these types of settings. Units of similar hydraulic conductivity properties have been termed hydrofacies (Poeter and Gaylord, 1990), and conceptual as well as mathematical models have been set forth that can capture the features of these settings (see Anderson, 1997). A hybrid approach to quantifying heterogeneity in a systematic fashion in settings containing hydrofacies changes is to delineate facies boundaries and within each facies consider a Gaussian hydraulic conductivity random field as characteristic of the facies. An example of this approach is illustrated by Tompson et al. (1999). Recent work emphasizes the need to numerically simulate observed patterns of geologic heterogeneity at the micro-scale and embed in patterns relevant to larger spatial scales that can be used as input to multi-scale transport models. Guin et al. (2010) and Ramanathan et al. (2010) provided an example of this approach to characterize physical heterogeneity for braided channel deposits.

Rehmann et al. (1999) used a spectral perturbation approach to couple the effect of geologic heterogeneity with virus transport at the field scale by considering aquifer hydraulic conductivity as a three-dimensional random field and evaluating the effects of spatial variability in aquifer hydraulic conductivity and virus transport parameters (attachment, detachment, and

inactivation) on large-scale virus movement. A stochastic mean model of virus transport was developed by coupling a system of local-scale, free-virus transport and attached-virus conservation equations with a random-field representation of aquifer and virus transport properties. The resultant mean equations for free and attached viruses were found to differ considerably from the local-scale (laboratory-scale) equations for homogenous media, and included effects such as a free virus effective velocity that differed from the velocity of a chemical tracer.

The work of Rehmann et al. (1999) was the first attempt to evaluate the effects of geologic heterogeneity on virus transport; however, the results were limited to periodic media (i.e., a spatial pattern that is repeatable over the path of the transport process) and to very large distances, on the order of 10–100 repeatable units of a geological pattern. This work was later extended to more general cases of geologic heterogeneity (no restrictions to periodic media) and without restrictions to very large travel distances (Maxwell and Welty, 2001; Maxwell et al., 2003; Maxwell et al., 2007). In a related effort, Sun et al. (2001) developed a two-dimensional numerical model of colloid transport in heterogeneous media where the coupling of physical heterogeneity to colloid filtration was explicitly incorporated, similar to the approach taken by Rehmann et al. (1999). Bhattacharjee et al. (2002) extended the work of Sun et al. (2001) to 2D virus transport in physically and chemically heterogeneous media.

4.3 Shallow Overland Flow and Transport

The conceptual picture of pathogen transport and fate during overland flow shown in Figure 4 provides a starting point to develop mathematical models to quantify these processes. Simplified forms of the Saint Venant equations (e.g., Abbott, 1979; Panday and Huyakorn, 2004) are typically used to describe overland flow. When the diffusion wave approximation is made (inertial terms are neglected in the momentum balance) then explicit expressions for depth averaged overland flow velocities can be obtained from the empirical, though well established, Manning water depth/friction discharge equation (DiGiammarco et al., 1996). It should be mentioned that proper choice of resistance parameters and flow characteristics are important considerations in models because overland flow can range from laminar to fully turbulent conditions depending on system conditions.

Equations describing subsurface water flow and pathogen transport were described in earlier sections of this review. The coupling of surface water and subsurface flow can be generally represented by interfacial exchange (infiltration and seepage), q_s (LT^{-1}), and earlier discussion indicates that this parameter is critical in determining pathogen removal during overland flow. Interfacial exchange is induced over a wide range of spatial scales,

producing very complex patterns of exchange that are difficult to parameterize in models (Wörman et al., 2007). In addition, most overland flow models do not consider the potential influence of depression and obstruction storage, and earlier discussion indicates that these properties are likely to have a large impact on pathogen transport and q_s . Panday and Huyakorn (2004) proposed several simple modifications to account for depression and obstruction storage during overland flow.

For large-scale groundwater flow models, q_s can be explicitly incorporated at the surface-subsurface interface using an analogy to Darcy's law (VanderKwaak, 1999). This type of macroscopic head difference formulation is an approximation for a suite of processes that regulate local exchange between overlying flow and pore water flow, and must be empirically parameterized at the scale of the model discretization. Alternatively, others have implicitly incorporated the surface water flow equation as a boundary condition for subsurface flow (He et al., 2008; Kollet and Maxwell, 2006). Various infiltration equations (Green and Ampt, 1911; Horton, 1940; Parlange et al., 1985; Philip, 1957) may also be used to quantify q_s when making simplifying assumptions about subsurface flow conditions. All of these formulations are highly approximate, as interfacial flow is a complex phenomenon and good strategies are not available for obtaining estimates of interfacial exchange for different levels of model discretization.

There are few models available for pathogen transport in overland flow that include interaction with the soil surface and underlying pore water. Generally the surface-pore water interaction in overland flow is treated similarly to deeper surface water flows, such as rivers. Most available models consider only solute transport, and just a few are available for colloid or pathogen transport. Transport models can be considered to fall within two major categories: (a) idealized models that provide an empirical or semiempirical parameterization of exchange between the water column and underlying soil/sediments; and (b) process-based models that explicitly represent the boundary geometry, flow configuration, and hydrodynamic processes.

Most idealized models do not explicitly consider the influence of depression and obstruction storage on flow and transport, but rather consider effective soil properties applied on idealized, smooth surfaces. These models generally follow one of three forms: diffusion models, interfacial boundary layer or mixing region models, and mass-transfer models. The most classic model assumes that the overlying flow is well mixed and that transport within the pore water is regulated by diffusion associated with the subsurface concentration gradient (Richardson and Parr, 1988). Some researchers have considered that infiltration water, runoff water, and soil water mix instantaneously in a thin mixing layer adjacent to the soil surface (Ahuja, 1982; Ahuja et al., 1981; Steenhuis and Walter, 1980). Interfacial diffusion models consider separate concentrations and mass balances in overland flow and the soil, and assume diffusive exchange between these regions through a

boundary layer due to the concentration gradient (Govindaraju, 1996; Wallach et al., 2001; Wallach and van Genuchten, 1990). This approach can also account for partial mixing of infiltration and runoff (Havis, 1986; Havis et al., 1992) and raindrop impact and erosion (Gao et al., 2004b; Gao et al., 2005). Alternatively, mass transfer models can be used, where interfacial transport is parameterized based on the difference in concentration between surface and subsurface reservoirs that are assumed to be well mixed (Bencala and Walters, 1983; Runkel, 1998).

Process-based models for surface-pore water interactions are not available specifically for overland flow, but some models developed for other flow configurations can potentially be applied to this problem. Interactions with regular, idealized geometries have been investigated as a way to develop general insight into governing mechanisms, suggesting that the modified Brinkman equations provide a good solution for coupling between pore fluid flow and laminar overlying flow (Rosenzweig and Shavit, 2007; Shavit et al., 2002). A variety of process-based models have been developed for surface-pore water interactions in rivers. Small-scale processes studied for rivers but relevant to overland flow include turbulent exchange fluxes, advective pore water flows induced by interactions between free-surface flow and boundary roughness, preferential flows associated with subsurface structure, and interactions between exchange flows and subsurface heterogeneity (Cardenas and Wilson, 2006; Cardenas et al., 2008; Elliott and Brooks, 1997a, 1997b; Higashino et al., 2009; Nagaoka and Ohgaki, 1990; Packman and Brooks, 2001; Zhou and Mendoza, 1993). The relative roles of pore water advection, diffusion, dispersion, preferential flow associated with sediment heterogeneity, and enhanced transport due to flow unsteadiness have also been investigated (Boano et al., 2010; Jin et al., 2010; Marion et al., 2008; Qian et al., 2007; Salehin et al., 2004; Sawyer and Cardenas, 2009). Information from many of the above studies and associated experimental observations has been synthesized into a proposed general scaling relationship for local exchange associated with grain-scale and roughness-related processes (O'Connor and Harvey, 2008).

More recently, efforts have been made to expand the types of models described above to include colloid and pathogen transport (e.g., Guber et al., 2009). Models for sediment transport in overland flow have considered deposition due to sedimentation (Wu, 2004). Again more has been done on shallow stream flow than on overland flow. Field injections of labeled particles has been interpreted in relation to transient storage models, suggesting that removal of fine particles from suspension depends on delivery to and retention in pore waters more than sedimentation (Karwan and Saiers, 2009; Newbold et al., 2005; Thomas et al., 2001). Models for pore water flow induced by flow-boundary interactions have been expanded to include particle settling and filtration (Packman et al., 2000; Ren and Packman, 2002), and also applied to analyze deposition of *C. parvum* oocysts (Searcy et al.,

2006b). Similar processes have been observed to occur in beds without large-scale roughness, suggesting that turbulence and/or grain-scale flows provide important mechanisms of particle delivery to pore water (Fries and Taghon, 2010). In all of these cases, colloid and pathogen deposition has consistently been observed to occur at a greater rate than attributable to sedimentation alone, indicating that advective delivery, pore water transport, and interactions with underlying sediments all contribute significantly to removal from suspension. Most of these studies have assumed steady deposition and homogeneous subsurface conditions. Ongoing colloid deposition-resuspension behavior associated with mobilization of sediments has been analyzed, indicating that colloids are readily remobilized along with coarser bed sediments, but that there can be longer-term accumulation of colloids below the region of active sediment transport (Packman and Brooks, 2001; Rehg et al., 2005). Recently, multiscale models have been developed that account for feedbacks between colloid deposition and permeability, leading to the development of heterogeneity associated with patterns of colloid delivery from the overlying water column (Chen et al., 2010).

Subsequently we describe a semiempirical model formulation that can account for many processes that influence pathogen transport during overland flow. This model employs the depression/obstruction storage concept illustrated in Figure 4. In this case, the depth-averaged mass balance equations (vertically integrated over the depth of the overland flow domain) for microbe transport in surface water and depression storage are given as

$$\frac{\partial (\varepsilon (h) h C_{sw})}{\partial t} + h \frac{\partial (\rho (h) S)}{\partial t} = \frac{\partial h J_x}{\partial x} + \frac{\partial h J_y}{\partial y} - q_{ds} + q_m + h B_{sw} \quad (40)$$

$$\frac{\partial (\varepsilon (h_{ds}) h_{ds} C_{ds})}{\partial t} + h_{ds} \frac{\partial (\rho (h_{ds}) S)}{\partial t} = q_{ds} - q_{pm} + h_{ds} B_{ds}. \quad (41)$$

here h [L] is the local water depth, h_{ds} [L] is the local water depth in the depression storage, C_{sw} [NL^{-3}] is the microbe concentration in surface water, C_{ds} [NL^{-3}] is the microbe concentration in depression storage, $\rho(h)$ is the density of the surface described in detail below, S [NM^{-1}] is the concentration of microbes on the surface of the soil and/or vegetation, J [$\text{NL}^{-2}\text{T}^{-1}$] is the pathogen flux, q_{ds} [$\text{NL}^{-2}\text{T}^{-1}$] is the net pathogen flux from surface water to depression storage, q_{pm} [$\text{NL}^{-2}\text{T}^{-1}$] is the net pathogen flux from depression storage to the porous medium surface, q_m [$\text{NL}^{-2}\text{T}^{-1}$] is source term that accounts for the net pathogen flux from manure to surface water, x [L] and y [L] are coordinate directions parallel to the mean land surface, and the subscripts x and y denote these directions on indicated parameters. The parameters B_{sw} [$\text{NL}^{-3}\text{T}^{-1}$] and B_{ds} [$\text{NL}^{-3}\text{T}^{-1}$] are the microbe source/sink term for surface water and depression storage, respectively, due to growth, death,

and inactivation. Expressions for B_{sw} and B_{ds} may be obtained in a manner similar to that for B_w described previously.

The value of $\varepsilon(b)$ [–] is an empirical function of porosity to account for the fact that only a fraction of the horizontal surface area at a given b may be in contact with the surface water because of depression and obstruction storage. A parabolic function has been used to describe $\varepsilon(b)$, which varies between 0 to 1 in regions with depression and obstruction storage, and is 1 in the absence of these factors (Panday and Huyakorn, 2004). The value of $\rho(b$ and $b_{ds})$ [ML^{-3}] denotes the density of the surface soil and/or vegetation and is given as

$$\rho(b) = (1 - \varepsilon(b)) \rho_S \quad (42)$$

where ρ_S [ML^{-3}] is the specific density of the vegetation, or the bulk density of the porous medium. When both vegetation and porous medium are present then ρ_S is an effective bulk density of a composite vegetation and porous medium mixture that may vary with b .

The values of J_x and J_y are given as

$$J_x = \varepsilon(b) \lambda_x \frac{\partial C_{sw}}{\partial x} - R_x(b) v_x C_{sw} \quad (43)$$

$$J_y = \varepsilon(b) \lambda_y \frac{\partial C_{sw}}{\partial y} - R_y(b) v_y C_{sw} \quad (44)$$

where λ [L] is the hydrodynamic dispersivity, v [LT^{-1}] is the depth average flow velocity determined from the Manning water depth/friction discharge equation (e.g., DiGiammarco et al., 1996), and $R(b)$ [–] is an empirical function of resistance. The dispersive and advective fluxes in overland flow are accounted for using the first and second terms on the right side of Equations [43] and [44], respectively. The value of $R(b)$ accounts for differences in conductance with b due to depression and obstruction storage (Panday and Huyakorn, 2004). The value of $R(b)$ is set equal to 0 when $b < b_{ds}$, and varies from 0 to 1 when $b > b_{ds}$ depending on the obstruction storage.

The parameter q_m accounts for microbe release from manure exposed to flowing water. Various functional forms have been proposed to describe this pathogen source (Pachepsky et al., 2006). Guber et al. (2006) reported that the model of Bradford and Schijven (2002) gave a superior description of microbe release from runoff experiments.

The mass balance equations for pathogen transport in soil were presented earlier in the review, and they are coupled to overland flow and depression storage through q_{pm} . The values of q_{ds} and q_{pm} account for exchange between overland flow, depression storage, and the soil surface due

to diffusion, infiltration/seepage, sedimentation, and raindrop impact as

$$q_{ds} = \varphi_1 (C_{sw} - C_{ds}) + q_s c^* + q_{sed} C_{sw} + E_r (\tau C_{sw} - C_{ds}) \quad (45)$$

$$q_{pm} = \varphi_2 (C_{ds} - C) + q_s c^\# + q_{sed} C_{ds} \quad (46)$$

where c^* [NL⁻³] is the microbe concentration equal to C_{sw} for infiltration and C_{ds} for seepage, $c^\#$ [NL⁻³] is the microbe concentration equal to C_{ds} for infiltration and C for seepage, φ_1 and φ_2 [LT⁻¹] are the exchange rates between the overland flow and depression storage and between depression storage and the soil, respectively, q_{sed} [LT⁻¹] is the microbe sedimentation velocity, E_r [LT⁻¹] is the rate of water ejection in the mixing zone to overland flow due to rainfall, and τ [-] is the fraction of the pathogen concentration entering the mixing layer from overland flow. The value of q_{sed} is given by Stokes settling velocity as follows when the microbe is spherical:

$$q_{sed} = \frac{2r_p^2(\rho_p - \rho_f)g}{9\mu_w} \quad (47)$$

where r_p [L] is the microbe radius, ρ_p and ρ_f [ML⁻³] are the microbe and fluid density, g [LT⁻²] is the acceleration due to gravity, and μ_w [ML⁻¹T⁻¹] is the water viscosity. The value of E_r is given as (Gao et al., 2004b):

$$E_r = \frac{ap\theta_w}{\rho_b} \quad (48)$$

where a [ML⁻³] is the pathogen detachability and p [LT⁻¹] is the precipitation rate.

The mass balance equations for microbes on the surface vegetation and/or porous medium in surface water and depression storage are given as

$$\frac{\partial (\rho (h) S)}{\partial t} = \varepsilon (h) k_r C_{sw} - \rho (h) k_d S + B_s \quad (49)$$

$$\frac{\partial (\rho (h_{ds}) S)}{\partial t} = \varepsilon (h_{ds}) k_r C_{ds} - \rho (h_{ds}) k_d S + B_s \quad (50)$$

where k_r [T⁻¹] and k_d [T⁻¹] are the first order rates of microbial retention and detachment from the solid/vegetation. The value of B_s (microbe sink/source term for surface water) was discussed previously for soil. However, large differences in B_s are expected for soil, surface water, and depression storage.

While complicated, the above model formulation uses effective, semiempirical parameterizations for pathogen transport in overland flow. Notably, the depression storage concept, like most mixing-zone concepts, represents an effective discretization of flow into a series of regions with distinct flow

and transport properties. In reality, there is a continuum of flow between the surface and subsurface, and the flow configuration changes continuously as water inputs change relative to boundary friction and pore water infiltration. Momentum transfer between free-surface and pore water flows, friction associated with flow around soil grains and over surface roughness, and connectivity across a wide array of topography are all complex, and as yet there are no general, fundamental models for these processes. The model presented above also did not account for many other processes that are known to influence pathogen transport. For example, interaction of pathogens with mobile soil grains and other particles in overland flow may substantially influence their mobilization and transport, as described previously in this review. In addition, subsurface flow models may need to account for evapotranspiration, preferential flow processes, and the effects of freezing and thawing on soil hydraulic properties in order to accurately simulate overland flow and associated pathogen transport. Hence, simulation errors may arise based on the selected conceptual framework, as well as on a lack of quantitative descriptions for the underlying processes. As a result, current models for pathogen transport in overland flow are limited to formulations that must be calibrated to site conditions and cannot be generalized to make independent predictions. Alternatively, probabilistic models for pathogen transport with overland flow may need to be developed (e.g., Guber et al., 2009) in analogy to methods discussed earlier for porous media.

4.4 Upscaling

As the complexity of the model increases, so does the number of required parameters. Many of these parameters are difficult to measure and quantify, and very limited databases are available in the literature to estimate relevant distributions for select environment conditions. Model parameters are likely to be determined from multiple types of measurements and at different scales (batch, column, and hillslope), and the results should therefore be interpreted cautiously. Further complications arise in determining transport and fate parameters in systems that are not representative of the terrestrial environments being modeled. In addition, many transport and fate parameters are known to exhibit large spatial and/or temporal heterogeneity. For example, pathogen concentrations and soil hydraulic conductivity may vary over many orders of magnitude across short distances in the field. Variability in soil hydraulic properties and soil topology will also influence simulation boundary and initial conditions. All of these factors will introduce large uncertainty in deterministic simulations at larger spatial scales.

Model input parameters are frequently measured on much smaller spatial and temporal scales than the simulation grid size and time step that is computationally efficient. This presents a challenge to determine effective

upscaled model parameters to obtain physically meaningful simulation results; especially when parameters vary significantly over short distances and time. Hence, modeling studies have been demonstrated to be sensitive to the grid size (e.g., Gupta et al., 2002). Scaling standards to determine effective parameters with different grid sizes may be possible, but have not yet been firmly established. Multicomponent, multimedia modeling represents a major challenge. It is not guaranteed that parameters for different constituents, such as water, solutes, inorganic colloids, and pathogens, will upscale similarly. As a result, it could potentially be extremely difficult to obtain compatible upscaled parameters needed to evaluate important interactions that are known to influence pathogen transmission. Furthermore, overland flow and transport is typically simulated at a much larger spatial and shorter temporal scale than in the subsurface, which can cause numerical challenges when the two domains are coupled. As the spatial scale of the domain increases, the simulation resolution may become limited by computation constraints. No recommendations currently exist about the grid size in modeling studies at the hillslope scale.

Despite the previously noted limitations, mathematical models serve as a distillation of current knowledge and our best means of generating predictions. Potential applications of mathematic models include predicting outcomes under given assumptions, testing hypotheses, identifying conditions and locations of increased risk, developing treatment strategies, and informing policy makers. Mathematical models may also be run in inverse mode to optimize flow and transport parameters to available experimental data. However, model calibration experiments are frequently based on results from simple grab samples that are analyzed for indicator microbes, and are not designed to determine important components of the microbe and/or water mass balances. It is unclear if typical grab samples will be representative of the sampling location as the spatial and temporal scale increases, and transport differences in indicator microbes and pathogens are very likely. All of these factors may lead to nonunique estimates of model parameters during calibration experiments. Use of models outside the range of calibration (e.g., other flow rates, locations, seasons, microbes) therefore should be done with awareness of the model validity.

Uncertainty in model inputs does not preclude the use of flow and transport simulations, but the probability density functions of all model parameters need to be determined or estimated. Multiple simulations can be run in a Monte Carlo framework to sample the input model parameter distributions (e.g., Guber et al., 2009). Information from the ensemble simulations can then be used to estimate the distribution of possible simulation outputs and the associated statistics (mean and variance) at select times and locations. Stochastic theories of subsurface flow and transport (Dagan and Neuman, 1997; Gelhar, 1993) provide a robust framework for upscaling spatial variability to large-scale properties and processes. In this approach, mathematical

perturbation techniques can be used to derive large-scale material property coefficients such as the hydraulic conductivity tensor and the hydrodynamic dispersion tensor, which are shown to be functions of the statistics of small-scale material properties (mean, standard deviation, correlation scales in three spatial directions). What remains a challenge is our ability to measure the small-scale properties required to determine the statistical inputs for the large-scale parameters. Stochastic theories can be used to design meaningful small-scale observations needed to parameterize the large-scale coefficients.

Pathogen transport and fate models are commonly used to address issues of public concern, such as total maximum daily loads (Shoemaker et al., 2005). However, it should be emphasized that a model is only as good as the validity of its assumptions and the quantification of required input parameters. Unfortunately, many implicit assumptions in pathogen transport and fate models in agricultural settings have not been rigorously tested. In addition, it is frequently not economically feasible to rigorously quantify all required model input parameters. In such cases, mathematical models should be viewed as an uncertain tool for management, instead of a precise predictor, and caution is warranted in interpreting their output. As increased knowledge and computational power becomes available, mathematical models need to be updated and refined to better reflect reality.

5. TREATMENTS TO REMOVE PATHOGENS

Laboratory, field, and modeling research discussed in previous sections have been carried out to increase the knowledge pool for designing scientifically sound policies and measures to avert pathogen contamination in agricultural settings. In addition to conventional treatment, vegetative strips and riparian zones can be used to attenuate the transport of pathogens. To date, it is still difficult to prevent the waterborne transmission of pathogens in agricultural settings given high pathogen loads, low infectious doses, long survival times, high spatial variability in surface and subsurface environments, and high temporal variability in water inputs and flow rates. As a result, multiple barriers are needed to reduce the amount of viable pathogens entering pathways and to mitigate the transport of pathogens in agricultural settings (Lang et al., 2007; Topp et al., 2009). Commonly used barriers include effective waste management to reduce pathogen loading, proper application of waste to lands to minimize pathogen movement to adjacent waters, and implementation of treatment measures (e.g., vegetative treatment areas and constructed wetlands) to control the pathogen transport (Oliver et al., 2007b). Infiltration ponds and galleries, riverbank and sand filtration systems have also been used to treat contaminated surface waters (Ellis, 1985; Hunt et al., 2002; Ray et al., 2002; Schijven and Hassanizadeh, 2000; Tufenkji et al., 2002; Weiss

et al., 2005). Considering the scope of this review, we limit detailed discussion to treatment measures such as vegetative treatment areas or buffer strips, and constructed wetlands because these widely used systems have water flow and pathogen transport components which can greatly benefit from the increased knowledge pool in the transport of pathogen in overland and subsurface flows as reviewed in previous sections.

Vegetative treatment areas are designed land areas with vegetation (e.g., perennial grass) that are widely used to control the transport of soil sediment, nutrients, and microorganisms from agricultural sources (Koelsch et al., 2006; Oliver et al., 2007b). Microbial retention by vegetative treatment areas has been variable in plot-scale studies with both simulated and natural rainfall events (Fajardo et al., 2001; Koelsch et al., 2006; Mawdsley et al., 1995; Pachepsky et al., 2006; Tate et al., 2004). This variability in performance is likely attributable to differences in design and operation of vegetative treatment areas, and incomplete characterization of factors influencing exchange with the surface mixing zone and soil. Schellinger and Clausen (1992) observed a minimal retention of fecal coliform and fecal streptococcus (only 0.16 log₁₀ reduction in concentrations), which they attributed to an excessive hydraulic loading rate. Other studies have shown that vegetative treatment areas could be an effective technique to reduce the loadings of coliforms to adjacent surface or subsurface waters if properly implemented (Lim et al., 1998; Tate et al., 2006); or protozoa (Atwill et al., 2002; Tate et al., 2004). It is generally believed that the efficacy of vegetative treatment areas is related to length, slope, soil type and structure, and vegetation cover (Atwill et al., 2002; Koelsch et al., 2006; Lim et al., 1998; Oliver et al., 2007b). However, Entry et al. (2000) also observed that the number of coliform cells did not continue to decrease as the applied wastewater moved downslope, indicating that there is possibly an optimal length of vegetative treatment areas beyond which no additional benefit of increased removal can be gained (Sullivan et al., 2007). Additionally, concentrated flow at the soil surface and preferential or macropore flows in the subsurface could reduce the effectiveness of vegetative treatment areas (Faulkner et al., 2011; Kim et al., 2006; Schellinger and Clausen, 1992). Therefore, the development of optimal design criteria is urgently needed for better utilization of this technique as a barrier to pathogen movement.

Riparian buffer strips function similarly to vegetative treatment areas, but are more critical because they are the last control point before the pathogens enter streams (Oliver et al., 2007b). Field and modeling studies suggest that riparian buffer strips could effectively hinder pathogen delivery to streams (Collins and Rutherford, 2004; Entry et al., 2000). However, complications arise from groundwater-surface interactions and bank erosion that may release significant numbers of pathogens to streams. At this time, the efficiency of riparian buffer strips remains uncertain (Collins and Rutherford, 2004;

Oliver et al., 2007b), and more research is needed to optimize performance of these systems.

Modeling of water and pathogen transport through vegetation will not only improve the design of barriers (Pachepsky et al., 2006), but also the prediction of pathogen movement at larger scales. Several empirical models are available for the performance of vegetative barriers, including GRASSF, SEDIMOT II, and the latest being VFSSMOD-W (Muñoz-Carpena and Parsons, 2010). Available models for the transport of microbes through the soil profiles and over the land surface were reviewed in previous sections. Limited effort has been made to couple the empirical VFSSMOD-W with pathogen transport (Zhang et al., 2001b), and more research is needed to develop models specifically for simulating pathogen transport.

Mechanistic modeling at the hillslope scale has also been explored (Kouznnetsov et al., 2007). Essentially, vegetative treatment areas are represented at the two-dimensional hillslope scale. The Saint Venant equations are used for surface runoff, and the Richards equation is used for subsurface flow. The surface and subsurface transport of microorganisms is modeled by an advection-dispersion equation with appropriate sink and source terms. Reasonable results have been obtained with this approach, which shows promise for further modeling of vegetative treatment areas in a more mechanistic manner based on experience with hillslope modeling. In addition, Faulkner et al. (2010) explored a water-balance model that includes soil water saturation in order to specify geometries and hydraulic loadings to avoid the risk of surface discharge that would lead to rapid pathogen export.

Constructed wetlands (CWs) are engineered ecological systems that utilize natural processes for water and wastewater treatment, and are now recognized as a viable technology to treat domestic wastewaters, treatment plant effluents, stormwater, and agricultural wastewaters (Cronk, 1996; Kivaisi, 2001; Vymazal, 2011). Mimicking natural wetlands, CWs consist of porous media and biota adapted to flooding or water clogging. Various configurations of CWs have been developed, including surface flow CWs, horizontal subsurface flow CWs, and vertical subsurface flow CWs (Langergraber, 2008; Truu et al., 2009; Vymazal, 2011). Surface flow CWs often have standing water above the substrate up to 0.6 m deep, whereas subsurface flow CWs have no visible surface water (Oliver et al., 2007b; Langergraber, 2008). While surface flow CWs historically have been popular in North America and Australia, horizontal subsurface CWs are more prevalent in Europe (Cronk, 1996; Kadlec, 2009; Vymazal, 2011). Nonetheless, a recent analysis showed that these two types of wetlands perform similarly (Kadlec, 2009).

Traditionally, CWs have been designed using criteria based on wastewater nutrient constituents (e.g., biological or chemical oxygen demands [BOD or COD], nitrogen, or phosphorus; Cronk, 1996; Rousseau et al., 2004). Properly designed and operated CWs have been shown to effectively reduce concentrations of BOD, COD, total suspended solids (TSS), nitrogen, and

phosphorus, as well as indicator or pathogenic microorganisms (Babatunde et al., 2008; Cronk, 1996; Kivaisi, 2001; Vymazal, 2011). Both biotic and abiotic mechanisms can contribute to removal of microorganisms, including predation, die-off, release of antibiotics by plants and other microbes, sedimentation, filtration, adsorption, chemical oxidation, and UV irradiation in the case of surface flow systems (Werker et al., 2002; Oliver et al., 2007b). A wide range of microorganism removal efficiencies has been reported (Kivaisi, 2001; Oliver et al., 2007b; Kadlec, 2009). Decamp and Warren (2000) reported a removal efficiency of 96.6–98.9% for *E. coli* in a pilot-scale horizontal subsurface flow CW. Morgan et al. (2008) found that after passing through a series of anaerobic, aerobic, and clarifier reactors and wetland cells, average total coliform and *E. coli* concentrations were decreased by at least 99% in dairy wastewater. In four surface flow CWs treating agricultural irrigation return flow in California, removal efficiencies were observed to be 66–91% for *E. coli*, and 86–94% for *enterococci* (Díaz et al., 2010). The removal of cysts of *Cryptosporidium parvum* and *Giardia lamblia* was reported to be 2 log in two pilot subsurface CWs for treating sewage wastewater with respective 10,000 and 300 population equivalents in Germany (Redder et al., 2010). Kadam et al. (2008) also reported 2–3 log reductions for a suite of indicator microorganisms and enteric pathogens in a vertical subsurface flow CW, and claimed that with extended recycling a removal of 5 log could be achieved. Chendorain et al. (1998) used MS2 coliphage as a model for human enteric virus and reported $97 \pm 3\%$ removal in the surface flow CWs, whereas Hodgson et al. (2004) determined that in a subsurface flow CW the removal of MS2 and *Enterobacter cloacae* phage was 2% and 9% in the winter, and 77% and 64% in the summer, respectively. This kind of variation occurs because of the vast differences in the CWs in terms of design, operation, wastewater characteristics, and environmental factors. This calls for a more rigorous design of CWs so that the removal goal of the CWs can be achieved for both nutrients and microorganisms.

CWs have been designed based on empirical regression equations, first-order decay models, Monod-type equations, or more complex dynamic and compartmental models (Langergraber, 2008; Rousseau et al., 2004). Surface flow CWs have generally been modeled with simplistic plug flow and the first-order kinetics (Cronk, 1996), whereas subsurface flow CWs have been modeled with more complex descriptions of water flow and reactive transport (Langergraber, 2008). However, CW designs have only rarely been explicitly based on the removal of pathogens (Cronk, 1996; Rousseau et al., 2004). This represents a significant knowledge gap if the CWs are considered to be employed as a barrier to mitigate pathogen export from agricultural sources to waterways. Improved understanding on the mechanisms of pathogen removal in CWs will support refined design of CWs for pathogen control. Basic research on pathogen transport in overland flow, saturated and

unsaturated subsurface flow are all relevant to CWs because of the various system configurations currently in use.

The design and implementation of treatment measures at the field scale requires fundamental knowledge of pathogen fate and transport at other scales. Significant progress has yet to be made to incorporate knowledge gained from smaller laboratory studies and field studies. Uncertainty arising from spatial and temporal heterogeneity in environmental parameters and in physicochemical characteristics of pathogens may be addressed through additional research that employs stochastic approaches. Properly designed and performed treatment measures can serve as critical control points in pathogen transport pathways in agricultural settings.

CONCLUSIONS

This review summarizes our current conceptual understanding and mathematical models for pathogen transport and fate in agricultural settings, and discussed potential treatments to mitigate risk. Subsequently we briefly highlight some of the main findings.

Determination of pathogen concentrations in environmental samples requires specialized equipment and personnel, and is therefore costly. In addition, current methods suffer from a number of limitations that may lead to significant underestimates or overestimates of pathogen concentrations. Consequently, measurements of indicator microorganisms are frequently used as surrogates for pathogens in the environment. However, little attention has been paid to potential differences in the transport and fate of indicators and pathogens, although pronounced differences are expected. Furthermore, the extraction efficiency of pathogens and indicators in environmental samples is frequently very poor, highly variable, and often times not reported. These analytical limitations make it difficult to quantitatively evaluate pathogen transport and fate in the environment. As a result, most studies to date have been undertaken in well-controlled laboratory conditions, and field-scale studies have mainly been limited to qualitative observations.

DLVO theory has commonly been used to characterize the adhesive interaction of pathogens with the SWI, AWI, and other colloids, even though this theory is thought to not be strictly applicable in most instances. In particular, cell surface macromolecules and structures, and extracellular polymeric materials produced by cells are now recognized to play potentially important roles in the interactions of pathogens with environmental surfaces. In addition, nano- to micro-scale heterogeneities on the SWI (cations, metal oxides, clays, and roughness) and pathogens may have a pronounced influence on adhesive interactions under unfavorable attachment conditions, rendering macroscopic surface characterizations of limited worth.

A wide variety of abiotic (e.g., temperature, attachment, and moisture content) and biotic (e.g., competition, predation, and biofilms) factors have

been demonstrated to substantially influence pathogen survival. However, most survival studies only consider a very limited set of conditions that may not always be representative of dynamic natural systems and the disparate environments that are encountered during transport. Consequently, survival during transport is exceedingly difficult to predict, and considerable uncertainty remains concerning survival in agricultural settings. The potential for long-term survival of persistent subpopulations, factors that control the regrowth of bacterial pathogens in the environment, transformations between active and vegetative phenotypes, and interactions between fecal pathogens and indigenous microbial populations are of particular concerns for pathogen transmission.

Pathogen retention in porous media depends on mass transfer from the bulk fluid to environmental surfaces and the balance of applied and resisting forces and torques that act on pathogens at these locations. Consequently, pathogen retention is a complex function of many factors (e.g., adhesion, water velocity, diffusion, grain size and distribution, pore structure, water content, and colloid size, distribution, and concentration). Retention under unfavorable attachment conditions has mainly been attributed to chemical heterogeneity (metal oxides, clays, and multivalent cations), and locations associated with lower fluid velocities (grain-grain contacts, surface roughness locations, AWS contact line, and dead end pores). Pathogen retention may be time-dependent as a result of blocking/filling of a limited number of retention locations or changes in microbial physiology following contact with the surface, and thus will depend on both the concentration of pathogens in suspension and the duration of transport. In addition, pathogen retention will be influenced by longer-interactions that lead to changes in pore structure, such as clogging. In general, much more is known about pathogen retention under (a) favorable than unfavorable attachment conditions; (b) saturated than unsaturated conditions; (c) in coarse, homogeneous sand compared with fine, graded, and structured soil; and (d) for homogeneous compared with heterogeneous suspensions.

Release of pathogens from dispersed manure sources to flowing water is a primary source of pathogens in agricultural settings. Pathogen detachment from soils and sediments is an important secondary source. Pathogen detachment depends on the system hydrodynamics that produces erosion, propels particles into solution, or applies a hydrodynamic torque that exceeds the resisting torque due to adhesion, gravity, and/or friction. Transients in solution chemistry and water content, which are common in the vadose zone, also induce pathogen detachment. In particular, detachment is enhanced when the adhesive interaction is reduced by a decrease in solution IS, when divalent cations are replaced by monovalent cations, and/or when the solution pH is increased. Transients in water content have also been reported to enhance colloid release due to expansion of water films and filling of pores that previously immobilized colloids, and scavenging of colloids from the AWI and AWS contact line, although these processes are not fully understood.

Pathogen transport in agricultural settings is strongly influenced by both subsurface and surface hydrologic processes, and interactions with many environmental surfaces. The level of complexity is dramatically enhanced by heterogeneity of soil hydraulic properties and surface topography, as well as by temporal variability in temperature, water inputs, and pathogen sources. Intense rainfall events can generate runoff and preferential flow that can rapidly transport pathogens over large distances. Pathogens that survive for extended periods of time have a greatly enhanced probability of being subject to such rapid-transport events. This can include organisms that have highly persistent environmentally transmissible forms, such as *C. parvum* oocysts, or persistent subpopulations. Conversely, in seasons of low rainfall and/or irrigation the transport and fate of pathogens is expected to be more greatly influenced by interactions with diverse environmental surfaces.

There is no consensus on the best model formulation for pathogen transport through soil and aquifer materials even for simple, well-defined systems. In fact, considerable model complexity has had to be invoked to account for the fact that retention profiles are commonly not exponential with depth, including multiple kinetic retention sites, two region models, and stochastic formulations. Similarly, pathogen survival is commonly modeled as a first-order decay process even though much research has indicated considerable deviation from this behavior and the potential for regrowth of bacterial pathogens. Most models and theory have been developed for homogeneous, saturated conditions, and do not consider the full physical, chemical, and biological complexities of dynamic natural systems. This is due in part to uncertainty in process descriptions, difficulty in parameter estimation and/or determination, incomplete characterization of heterogeneity at a variety of spatial and temporal scales, and the strong coupling of processes with a wide variety of factors (i.e., water flow and content, temperature, nutrients, and colloid concentration). Consequently, there remain many challenges to determine effective upscaled model parameters for physically meaningful large-scale simulations, and no scaling standards have been firmly established.

A variety of models also describe pathogen transport and fate in overland flow. However, most of these models do not account for realistic interactions with the ground surface and underlying pore water due to depression and obstruction storage, but rather consider effective soil properties and idealized, smooth surfaces. Furthermore, overland flow and transport is typically simulated at a much larger spatial scale and shorter temporal scale than subsurface transport, leading to substantial numerical challenges in coupling transport in the two domains. The depression storage concept discretizes flow into a series of regions with distinct flow and transport properties. In reality, there is a continuum of flow between the surface and subsurface, and the flow configuration changes continuously as water inputs change

relative to boundary friction and pore water infiltration. There are no fundamental models for these processes. Numerous process-based models have been developed to simulate surface-pore water interactions in rivers, and these can potentially be adapted for pathogen interactions with soils during overland flow. However, additional complexities are expected due to variably saturated flow conditions, preferential flow processes, raindrop impact, erosion, evapotranspiration, and the effects of freezing and thawing on soil hydraulic properties. In addition, interaction of pathogens with mobile soil grains and other particles in overland flow may substantially influence their mobilization and transport.

Mathematical models represent condensed summaries of current knowledge and are our best means of predicting pathogen transport and fate in agricultural settings. Potential applications of mathematic models include predicting outcomes under given assumptions, testing hypotheses, identifying conditions and locations of increased risk, developing treatment strategies, and updating policy decisions. However, it should be emphasized that a model is only as good as the validity of its assumptions and the quantification of required input parameters. Mathematical models currently should be viewed as an uncertain tool for management, instead of a precise predictor, and caution is warranted in interpreting their output. Additional research is needed to better account for uncertainty in model parameters and predictions.

Multiple barriers are needed to reduce the number of viable pathogens transported in agricultural settings. Commonly used strategies to reduce pathogen export include waste management to reduce pathogen loading, application of waste to lands to minimize pathogen movement to adjacent waters, and installation of physical barriers to pathogen transport. Infiltration ponds and galleries, and riverbank filtration systems have been used to treat contaminated surface waters. Vegetative and riparian treatment areas, buffer strips, and constructed wetlands have also been used to remove pathogens. However, the efficiency of these systems is difficult to predict owing to differences in design and operation, and incomplete characterization of factors influencing exchange with vegetation stands and underlying soils and sediments. Better understanding of the underlying pathogen transport and removal processes in surface and subsurface waters is needed to improve the design of efficient barriers.

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