Release Rates of Manure-Borne Coliform Bacteria from Data on Leaching through Stony Soil

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ABSTRACT

Manures are sources of several human pathogens that can potentially contribute to surface and groundwater contamination. Microorganisms must first be released from the manure matrix before they can infiltrate into and leach through the vadose zone. The objective of this study was to estimate rates of rainfall-induced release of fecal coliform (FC) from surface-applied bovine manure. Simulated rainfall of 7.1 cm h^{-1} was applied to the surface of 90-cm-long lysimeters filled with the undisturbed stony soil. When the steady state was reached, clumps of manure were placed on the surface. Rainfall was continued for about 5 h after application of manure, and 10-min leachate portions were analyzed for turbidity and FC. The convective–dispersive equation with linear adsorption–exclusion and the first-order removal–regrowth terms was used as a model of the coliform transport in soil. Asymptotic properties of the solution of this equation with the exponentially decreasing boundary concentration were used to infer the release rate constant from the FC breakthrough curves. A value of 0.0054 ± 0.0015 min^{-1} was found for the FC release rate constant. The regression line of reduced coliform concentrations on reduced turbidity values was not significantly different from the one-to-one line; R^2 was 0.807. Assuming that turbidity can be used as a measure of concentration of manure particulates in leachates, we found that average values for the release rate constants were not significantly different for FC and manure particulates. The average velocity of bacteria and manure particulates transport was about seven times larger than the average pore velocity. The proposed technique of estimating FC and manure release rates shows promise for use in further studies needed to elucidate and assess factors affecting release rate.

There is growing concern regarding the potential for contamination of surface and ground water by pathogens from bovine manures. Even though they are considered to be a beneficial fertilizer and soil amendment, bovine manures are a substantial agricultural source of several human parasites/pathogens. *Escherichia coli* O157 and other EHEC strains are commonly found in beef and dairy cattle (*Bos taurus* L.) (Elder et al., 2000; Hancock et al., 1998; Porter et al., 1997). On-farm monitoring of *E. coli* O157:H7 suggests that shedding occurs episodically (up to 10^5 organisms g^{-1} feces) and can persist for variable periods of time ranging from about 1 to 5 mo (Shere et al., 1998; Zhao et al., 1995).

Pathogens applied onto soil surfaces may enter the soil and travel through the vadose zone until they reach groundwater (McMurry et al., 1998). A substantial body of literature exists describing leaching of microorganisms through soil. Early studies documenting subsurface bacterial transport from septic effluents were reviewed by Hagedorn et al. (1981) and Bitton and Harvey (1992). These studies describe bacterial transport from a few meters to 830 m, depending on soil or sediment texture and permeability, water saturation degree, and length of time. More recent studies have quantified the leaching potential of fecal bacteria and genetically modified bacteria. Although leaching rates were influenced to some extent by all soil and microbial properties, the predominant factors in disturbed soil cores appear to be soil structure and texture and porosity and bulk density, in conjunction with bacterial size (Gannon et al., 1991a,b; Huysman and Veerstraete, 1993; Tan et al., 1991). In intact soil cores, distribution and continuity of macropores (preferential flow pathways) in conjunction with initial water content appeared to be the predominant factors (Abu-Ashour et al., 1998; McMurry et al., 1998; Paterson et al., 1993). In general, higher leaching rates were observed in undisturbed soils as compared with disturbed soils (Smith et al., 1985; van Elsas et al., 1991), presumably due to the destruction of macropores during the repacking of soil.

The majority of studies of pathogen bacteria transport in soils used bacterial cells that were suspended in solutions and then applied to soil surface or mixed with the influent solution (Bitton et al., 1974; Abu-Ashour et al., 1998; Devare and Alexander, 1995; Goldschmid et al., 1973; Gannon et al., 1991a,b; Germann et al., 1987; Hendry et al., 1999; Hekman et al., 1995; Huysman and Verstraete, 1993; Hornberger et al., 1992; Lahlou et al., 2000; Paterson et al., 1993; Smith et al., 1985; Tan et al., 1991; van Elsas et al., 1991). Results of those studies are only partly applicable to microbial leaching from either land-applied or naturally deposited manures, since microorganisms must first be released from the manure matrix before they can infiltrate into and leach through the soil profile. We are not aware of any data on bacteria release rates from manure subject to rainfall, and could find only one recent paper (Bradford and Schijven, 2002) that contains data on manure dissolution in laboratory conditions. It is not known whether rates of manure dissolution and bacteria release can be equated since the bacteria distribution within manure material has not been studied. At the same time, knowledge of the release rates is a precondition of bacteria and manure transport simulations.

The objective of this study was to estimate rates of fecal coliform and manure release due to rainfall action.

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Abbreviations: CFU, colony forming units; FC, fecal coliform; NTU, nephelometric turbity units.

We obtained breakthrough curves of bacteria and manure particulates from soil columns and used the analytical solution (van Genuchten, 1985) of the convective–dispersive equation with exponentially decreasing inlet concentration to derive the release rates from tail sections of the breakthrough curves.

MATERIALS AND METHODS

Experimental

The lysimeters were located at the USDA-ARS experimental research site near the town of Leck Kill, PA. The soil in lysimeters is a loamy-skeletal, mixed, active, mesic Typic Hapludult. This soil is channery; that is, it has rock fragments of shale or thin flat sandstone or siltstone up to 20 cm long. Illite and kaolinite are the most common clay minerals. This is a deep, well-drained soil with rapid permeability. Percolation is dominated by the macropore flow. Soil properties are given in Table 1 (Stout et al., 1998). Lysimeters were 60 cm in diameter and 90 cm long. They were built according to the design of Moyer et al. (1996) and had a steel plate welded to the bottom with about twenty 6-mm holes in the middle 75 mm of the bottom. A 1-mm wire mesh was placed over the holes and about 4 kg of coarse gravel was placed over the mesh. The middle of the bottom of the soil core was hollowed out to accommodate the gravel. Detailed descriptions of the site and monolith lysimeters can be found elsewhere (Gburek and Folmar, 1999; Stout et al., 1998).

Rainfall was generated with an artificial rainfall simulator using a TeeJet 1/2 HH SS 50 WSQ nozzle (Spraying Systems Co., Wheaton, IL) placed approximately 305 cm above the soil surface. Rainfall was delivered at approximately 7 cm h^{-1} and had a coefficient of uniformity greater than 0.83 within the 2 by 2 m area directly below the nozzle. This intensity roughly corresponds to a 5-yr return period storm in central Pennsylvania. The rainfall simulator allowed for coverage of six lysimeters at one time used as replications. During the experiment, (i) rainfall was simulated over lysimeters until leachate occurred, (ii) time zero samples were collected from each lysimeter, (iii) rainfall was stopped and manure (2 kg lysimeter^{-1}, based on 70 000 kg ha^{-1} application rate) was applied to the surface of lysimeters, (iv) rainfall simulation resumed, and (v) leachate samples were collected at 10-min intervals (~20 mL) for 5 h.

Manure was collected from the lot of a local dairy farm on the previous day. Percentage dry weight was 28%. Manure was applied to the lysimeter surfaces in 10 to 12 lumps approximately in nodes of a grid.

Leachate samples were refrigerated continuously (4°C) until analysis, except during transport from Leck Kill, PA to Beltsville, MD when samples were packed in ice. Microbial analyses were initiated the next day after transporting samples to the laboratory and required 3 d (two lysimeters per day). Samples were plated on MacConkeys Agar using an Autoplate 4000 spiral plater (50 μL) manufactured by Spiral Biotech (Bethesda, MD). Total FC colony forming units (CFUs) were counted using a Protocol plate reader (Synoptics, Cambridge, UK). Turbidity (nephelometric turbity units, NTU) was determined using a Model 965-10A Turbidimeter manufactured by Orbeco Analytical Systems, Inc. (Farmingdale, NY).

Data Analysis

The FC transport was simulated assuming steady state water flow and neglecting variations in water content and pore structure along the soil profile. The model was chosen in the form

$$\frac{\partial}{\partial t}\left(\theta_a c + \rho s\right) = \frac{\partial}{\partial x}\left(\theta_a D \frac{\partial c}{\partial x} - J_w c\right) - \mu_l \theta_a c - \rho \mu_s s \quad [1]$$

Here c is the volume-averaged bacteria concentration in the soil solution (cells cm^{-3}), θ_a is the porosity available for bacteria transport in soil ($m^3\ m^{-3}$), ρ is soil bulk density (g cm^{-3}), s is the adsorbed amount of bacteria (cells g^{-1}), D is the dispersion coefficient ($cm^2\ min^{-1}$), J_w is the volumetric water flux density (cm min^{-1}), μ_l and μ_s (min^{-1}) are first-order decay coefficients to simulate removal of bacteria from solution and solid phase as a result of deposition and entrainment (Hornberger et al., 1992). If a regrowth occurs, values of μ_l and μ_s also include growth rate constants with negative signs.

Bacteria adsorption by the solid phase is described with a linear isotherm as

$$s = K_d c \quad [2]$$

where K_d is a distribution constant ($cm^{-3}\ g^{-1}$). Using Eq. [2], one rewrites Eq. [1] as

$$R \frac{\partial c}{\partial t} = \frac{\theta_a}{\theta} D \frac{\partial^2 c}{\partial x^2} - v \frac{\partial c}{\partial x} - \mu c \quad [3]$$

where $v = J_w/\theta$ (cm min^{-1}) is the average pore-water velocity, θ ($cm^3\ cm^{-3}$) is the volumetric soil water content, R_a is the retardation factor given by

$$R = \frac{\theta_a}{\theta} + \frac{\rho K_d}{\theta} \quad [4]$$

and μ is the combined first-order removal rate coefficient:

$$\mu = \mu_l \frac{\theta_a}{\theta} + \frac{\rho K_d \mu_s}{\theta} \quad [5]$$

The third-type boundary condition was used for the bacteria flux on the surface:

$$\left(J_w c - \theta_a D \frac{\partial c}{\partial x}\right)_{x=0} = J_w c_m(t) \quad [6]$$

The concentration of bacteria released from manure c_m (cells cm^{-3}) was assumed to decrease exponentially with time:

Table 1. Selected soil properties.

Horizon	Depth range	Coarse fragments 7.5–25 cm	Textural class	Permeability	Available water holding capacity† between 0.03 and 1.5 MPa	pH	Organic matter	CEC
	cm	g kg^{-1}		cm h^{-1}	$cm^3 cm^{-3}$		%	me/100g
Ap	0–20	150	Silt loam	1.5–15.2	0.1–0.14	5.0	2.0	0.16
Bt1	20–35	200	Silt loam	1.5–15.2	0.06–0.1	5.0	0.3	0.11
Bt2	35–75	300	Silt loam	1.5–15.2	0.06–0.1	5.0	0.3	11.0
C	75–90	400	Silt loam	1.5–15.2	0.06–0.1	5.0	0.3	11.0

† Measured onsite (Stout et al., 1998); other properties are derived with the MUUF software (Baumer et al., 1994).

$$c_m(t) = c_0 \exp(-\lambda t) \quad [7]$$

where c_0 is the initial concentration (cells cm^{-3}) and λ (min^{-1}) is the bacteria release rate constant.

Using dimensionless variables $C = c/c_0$, $Z = x/L$, $T = vt/L$, $P = vL\theta/(D_a\theta_a)$, $\mu^E = \mu L/v$, and $\lambda^E = \lambda L/v$, Toride et al. (1999) presented the solution of the boundary problem (Eq. [3]–[7]) for the infinite domain with zero initial concentration for arbitrary X and T values:

$$C(X, T) = \exp(-\lambda^E T) G_1^E(X, T, \mu^E - R\lambda^E) \quad [8]$$

where function G_1^E is

$$G_1^E(X, T, \Omega) = \frac{1}{1+u} \exp\left[\frac{P(1-u)Z}{2}\right] \operatorname{erfc}\left(\frac{RZ - uT}{\sqrt{4RT/P}}\right) + \frac{1}{1-u} \exp\left[\frac{P(1+u)Z}{2}\right] \operatorname{erfc}\left(\frac{RZ + uT}{\sqrt{4RT/P}}\right) - \frac{2}{1-u^2} \exp\left[PZ + \frac{P(1-u^2)T}{4R}\right] \operatorname{erfc}\left(\frac{RZ + T}{\sqrt{4RT/P}}\right) \quad [9]$$

with $u = \sqrt{1 + 4\Omega/P}$. This expression is valid when $\Omega > -P/4$ (i.e., $\mu^E > R\lambda^E - P/4$ and $\Omega \neq 0$). There exists also a solution for relatively small deposition and/or entrainment rates when $\mu^E < R\lambda^E - P/4$ (van Genuchten, 1985). This solution is expressed in the form of integrals that need to be evaluated using quadratures.

Using values of the erfc function at infinity, one can show that when T becomes large, the function $G_1^E(1, T, \Omega)$ given by Eq. [9] approaches the limit $2\exp[P(1-u)/2]/(1+u)$. Therefore the asymptotic behavior of concentrations for large T is

$$c \approx c_0 \exp(-\lambda^E T) \frac{2}{1+u} \exp\left[\frac{P(1-u)}{2}\right] \quad [10]$$

or

$$\log_{10} c \approx \log_{10}\left\{\frac{2c_0}{1+u} \exp\left[\frac{P(1-u)}{2}\right]\right\} - \frac{\lambda t}{\ln 10} \quad [11]$$

Therefore breakthrough curves obtained from the solution (Eq. [9]) have their tails well approximated with straight lines in the $\log_{10}$(concentration)–time coordinate plane. Slopes of those lines are values of the release rate constant λ divided by $\ln 10 \approx 2.303$.

We could not find an analytical expression for the asymptotic behavior of concentrations for the case of small μ^E values, when $\mu^E < R\lambda^E - P/4$ and values of u become complex numbers. However, multiple experiments with computing G_1^E by numerical evaluation of integrals using the subroutine *F* from the computer code given by van Genuchten (1985) showed that the loglinear asymptotic behavior of the breakthrough curves exists also for $\mu^E < R\lambda^E - P/4$.

Figure 1 shows examples of the breakthrough curves computed from Eq. [9] with sets of parameters that are realistic for the experiment in this work. The length L = 90 cm and the pore velocity v = 0.5 cm min^{-1} were used in simulations. Figure 1a shows that large (5–50 cm^2 min^{-1}) dispersion coefficient values will delay the beginning of the linear decrease of the $\log_{10}(c)$ with time. Figure 1b shows that the linear decrease begins soon after the minimum is reached for values of the retardation coefficient R less than one (i.e., when only part of the water-filled pore space is available for the solute transport). The effect of the release rate constant value on the concentration–time dependencies is shown in Fig. 1c. The FC concentration on the surface decreases very slowly when the release rate is small (0.001 min^{-1}), and this is reflected in the shape of the breakthrough curve showing almost constant concentration as the time progresses. The effect of the deposition–entrainment rate constant μ on effluent concentration is shown in Fig. 1d. An increase in value of μ causes vertical shift of the linear part of the breakthrough curve and does not affect slope of this linear part.

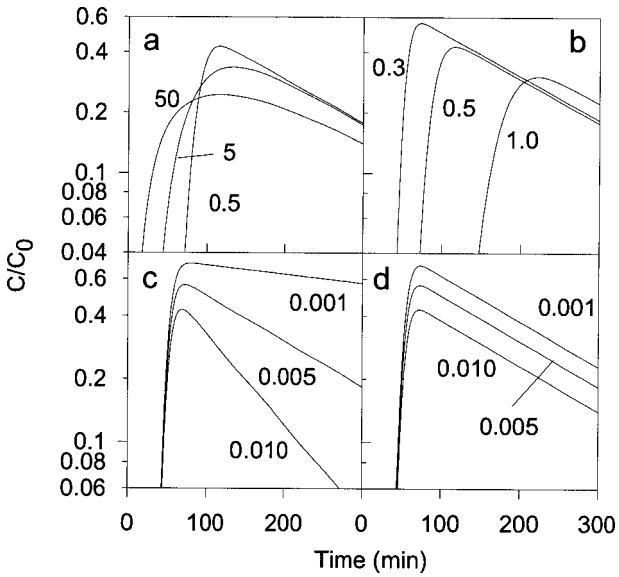

Fig. 1. Effect of transport and release parameters on simulated fecal coliform concentrations in effluent collected at 90 cm depth; values of the varied parameter are shown at corresponding curves. (a) Various D in cm^{2}min^{-1}, R = 0.5, μ = 0.005 min^{-1}, λ = 0.005 min^{-1}; (b) D = 0.5 cm^{2}min^{-1}, various R, μ = 0.005 min^{-1}, λ = 0.005 min^{-1}; (c) D = 0.5 cm^{2}min^{-1}, R = 0.3, μ = 0.005 min^{-1}, various λ in min^{-1}; (d) D = 0.5 cm^{2}min^{-1}, R = 0.3, various μ in min^{-1}, λ = 0.005 min^{-1}; pore water velocity v was 0.5 cm min^{-1} in all simulations.

The Student's t test with the significance level of 0.05 was used to test statistical hypotheses about the difference between regression slope and one, and between the regression intercept and zero for linear portions of log-transformed breakthrough curves at late times. The symbol "±" is used to separate average values from standard errors.

RESULTS

Data on FC in and turbidity of the effluent are shown in Fig. 2. Maximum FC concentrations were observed between 60 and 80 min. All FC breakthrough curves had a period of a steep increase followed by the gradual decrease phase.

Visible similarity in FC and turbidity breakthrough curves after 30 min of experiment suggested a relationship between the two variables. To observe this relationship, average effluent concentration during the experiment was calculated for each lysimeter, and reduced FC concentrations were computed by dividing actual concentrations by the average effluent concentration. Reduced turbidity values were obtained similarly. Dependencies of the reduced FC concentration and reduced turbidity on time are shown in Fig. 3a and 3b, respectively. Differences among the replications are much smaller for reduced values than for the original breakthrough curves in Fig. 2. The relationship between the reduced turbidity and the reduced FC concentration is shown in Fig. 3c. The estimated slope and intercept

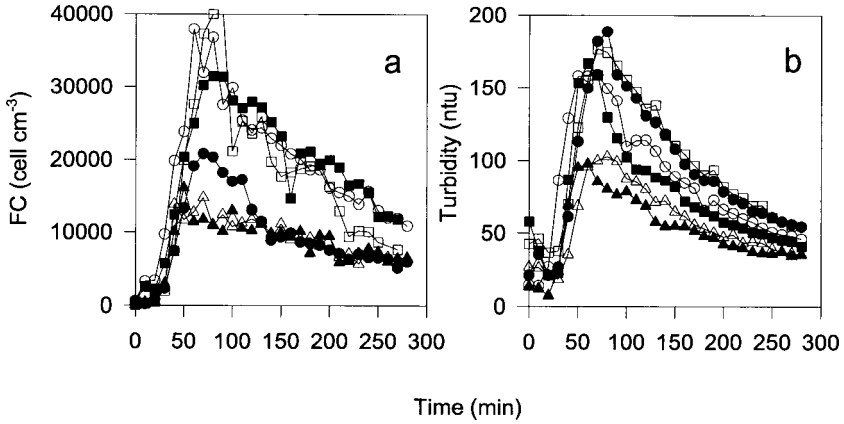

Fig. 2. Measured concentrations of fecal coliforms and turbidity; different symbols are used to distinguish among replications.

values do not significantly differ from unity and zero, respectively.

Dependencies of the logarithm of the FC concentration on time are shown in Fig. 4. The decrease after reaching maximum is approximately linear in all replications in accordance with the prediction of Eq. [11]. Figure 4b shows the intervals of the linearity after 110 min of the experiment. The regression lines shown in Fig. 4b had the determination coefficients R^2 varying mostly between 0.82 and 0.98 except one dataset that had R^2 of 0.698. The average slope of the six regression lines is -0.00234 ± 0.00063 min^{-1}, and therefore the FC release rate constant $\lambda = 0.0054 \pm 0.0015$ min^{-1}. The average half-time for the release of FC from manure was $\ln(2)/\lambda \approx 128$ min.

Assuming that (i) the transport of manure particulates can be simulated with the same mathematical model (Eq. [3]–[7]) as the transport of fecal coliforms, and (ii) turbidity is proportional to the concentration of manure particulates in effluent, we plotted data on turbidity in the $\log_{10}$(turbidity)–time coordinates (Fig. 4c). A well-expressed linearity of the graphs was observed after the maximum was reached. Figure 4d shows regression lines. Regressions had R^2 between 0.95 and 0.99, the average slope was -0.00231 ± 0.00028 min^{-1}. Therefore, the release rate constant for manure particulates λ_{mp} was 0.0053 ± 0.0006 min^{-1}. The average value was very close to and not statistically significantly different from the average FC release rate constant 0.0054 min^{-1}.

Although the accurate estimation of parameters in the model (Eq. [3]–[7]) is possible only if the initial FC concentration is known in the very first influent portion, a crude estimation of values of the transport parameters D and R can be made from comparison of graphs shown in Fig. 1 and 4a. Figure 1a shows that the initial steep concentration increase, as observed in Fig. 4a, corresponds to values of D about 0.5 to 1 cm^{2}min^{-1}. For such values of D, the graph in Fig. 1d shows that the entrapment–entrainment rate μ does not have much of an effect on the time at which the breakthrough curve begins the steep rise, and the graph in Fig. 1b shows that this time is approximately equal to RL/v, where L is the length of the monolith and v is the average pore-water velocity. The steep rise in Fig. 4a begins around 20 to 40 min; the average v value is about 0.42 cm min^{-1}. Therefore value of the retardation factor R appears to be in the range from 0.09 to 0.19.

DISCUSSION

Bacteria and manure particulates moved relatively fast through the soil profile in this work. The pore water velocity was seven times less than the average velocity of bacteria transport. This ratio of velocities is given by the value $1/R$, where R is the retardation coefficient that was about 0.15 in this work. Bitton and Harvey (1992) reviewed the values of retardation coefficient found in studies of *E. coli* in soil and groundwater and reported

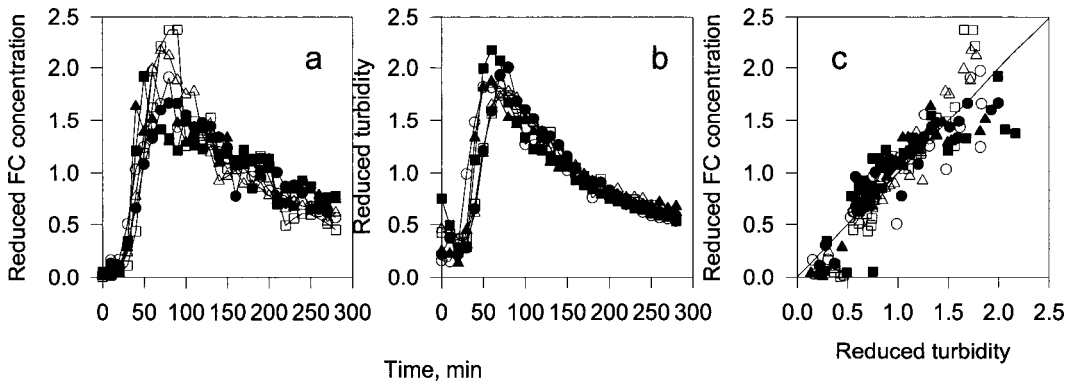

Fig. 3. (a) Reduced fecal coliform concentrations, (b) reduced turbidity of the effluent, and (c) relationship between the two reduced values. Different symbols are used to distinguish among replications. The linear regression line is shown in the Part c; the regression equation is $Y = (0.994 \pm 0.039)\ X + (0.003 \pm 0.042)$, $R^2 = 0.807$.

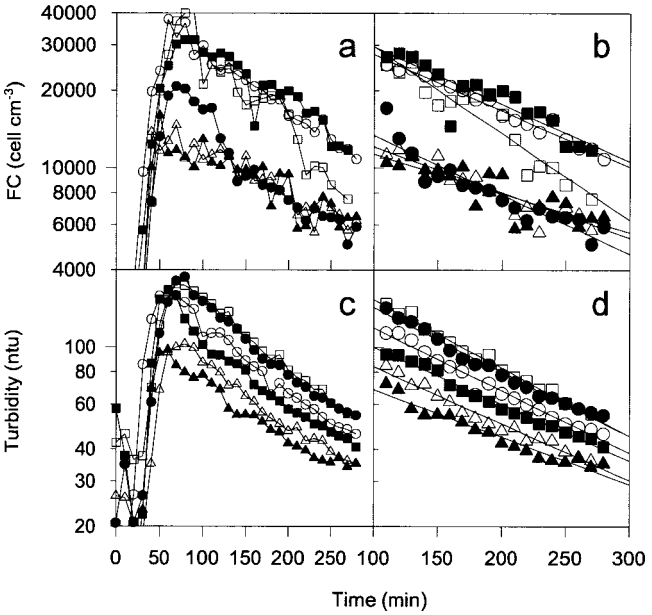

Fig. 4. (a), (b) Dependencies of logarithms of fecal coliform concentrations in the effluent, and (c), (d) logarithms of turbidity of the effluent on time: (a) and (c) complete observed dependencies, (b) and (d) parts of the observed dependencies used to derive release rate constant values as absolute values of the linear regression lines. Different symbols are used to distinguish among replications.

the range from 0.1 to 1.0. These authors attributed the apparent accelerated bacteria transport to the preferential transport of microorganisms along preferred flow paths (large pores, fractures and channels) because bacteria may be excluded from the smaller pores on the basis of their size. Values of the retardation coefficient R as given by Eq. [4] reflect both the unavailability of the part of pore space for the bacteria transport (first term) and the delay in bacteria transport caused by the adsorption on soil particles (second term). It is possible that bacteria adsorption on soil particles did not affect the bacteria transport in this work because this adsorption was slow. Bengtsson (1995) reported time scales to reach adsorption equilibrium for 13 bacteria species from 10 to 15 h to several days. Assuming the small effect of the adsorption on soil particles, we could estimate the porosity available for bacteria transport as 15% of the total water content.

Manure particulates composed of microbial biomass and partially digested food were transported along with FC. Using reduced values enhanced the similarity between dependencies of the effluent turbidity and the FC concentrations on time (Fig. 3), probably because reduced concentrations eliminated the effect of the initial concentration of FC and particulates on the breakthrough curves. The release and transport models for particulates apparently could be the same as for FC, and that assumption led to statistically indistinguishable release rate constants for manure particulates and for FC. Observed FC concentrations in leachate samples would not give a measurable turbidity. Consequently, turbidity was due to non-FC microbial biomass and other particulates. A relatively strong correlation between reduced FC concentrations in effluent and the effluent turbidity makes turbidity a plausible candidate for an easily measurable proxy variable to estimate the level of bacteria concentration in leachates. This correlation also raises the question of whether the transport bacteria released from manure to soil can be a facilitated transport. Manure amendment has been shown to affect transport of bacteria through soil (Gagliardi and Karns, 2000). Jin et al. (2000) presented the first experimental example of colloid-facilitated virus transport in sandy columns. It might be important to find out what percentage of bacteria travels in soil solution being attached to manure particulates.

An interesting feature of the FC transport in this experiment was a very low longitudinal dispersion. Fecal coliform transport developed almost as if it followed piston flow or a kinematic wave (Germann et al., 1987). Pore water velocities obviously did not have substantial variability in the pore space available for the bacteria transport, and that might be a consequence of the presence of manure material in solution affecting the soil water viscosity and density. Low dispersion allowed the asymptotic regime (Eq. [11]) to appear relatively early in the experiment, and we had enough data to estimate the release rate constants as shown in Fig. 4b and 4d.

Using properties of the analytical solution (van Genuchten, 1985) allowed us to obtain the release rates while avoiding the need of knowing the initial concentration c_0 in Eq. [7] for both FC and manure particulates. Measurements of the initial concentration in the experimental setup of this work would be very uncertain. We note that intercepts of the regression equations (Eq. [11]) are not suitable to compute the c_0 value because those intercepts include the value of $u = \sqrt{1 + 4(\mu^E - R\lambda^E)/P}$, which depends on the removal–entrainment rate constant μ^E. Data in Fig. 1d illustrate this point. Extending the linear sections of the breakthrough curves to the concentration axis leads to values of c/c_0 less than one.

We note that FCs represent only a small fraction of total microbial biomass in manure. It remains to be seen whether release and transport of bacterial pathogens (*Salmonella*, *E. coli* O157) as well as release of protozoan parasites, such as *Cryptosporidium* and *Giardia* can be mimicked by FC.

The exponential release model (Eq. [7]) performed reasonably well both for fecal coliforms and for manure particulates (Fig. 4). The release rate constants should depend on manure source, composition and management, as well as on rain parameters. The values of 0.005 min^{-1} found in this work should be treated as estimates applicable to this experiment but by no means the absolute values. We computed the manure release rates from the data of Bradford and Schijven (2002) and found values ranging from 0.002 to 0.003 min^{-1}. The difference between release rates in this and Bradford and Shijven's works may be related both to the differences in water application to manure and to the quality of manure used. Because knowledge of microorganism release rates is essential for simulations of pathogen dissemination from manures, further studies are needed to elucidate manure and microorganism release factors. Such studies may take

advantage of the promise shown by the breakthrough-based technique developed in this work.

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