VIRUS TRANSPORT THROUGH PERCOLATING BEDS

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ABSTRACT

The movement of viruses in soil has important implications for land treatment of waste water. An adsorption mass transfer model is developed to describe the movement of single virus particles through packed beds of soils and soil components. The model predicts that ϕx-174 bacteriophage will breakthrough one meter of silt loam soil in 60 days for percolation rates of about 40 in/wk. Experimental determinations of equilibrium adsorption parameters for particular virus/adsorbent combinations are required to make similar predictions for enteric virus breakthrough from soil columns. Experimental measurements for attenuated poliovirus I (a typical enteric virus) show that: (1.) single virus particles are likely to be present in treated wastewaters that are land spread, (2.) poliovirus association with sand is about 50-fold stronger than ϕx-174 phage association with silt loam soil, and (3.) poliovirus interaction with 1-2 μm montmorillonite clay particles is affected by clay aggregation at high virus titers. The needs for future research are indicated. These include measurements of enteric virus inactivation in soil-water and incorporation of inactivation kinetic expressions in future versions of the adsorption mass-transfer model.

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I. INTRODUCTION

Growing interest in various forms of water-reuse, and increased awareness concerning public health aspects of wastewater disposal require better understanding of the fate of human pathogens contained in domestic raw sewage. These pathogens include enteric viruses, bacteria, parasitic helminths and protozoa. Enteric viruses which originate primarily from human and animal feces, are of particular concern because of large uncertainty regarding the disease risk they represent when transmitted by water, their refractory response to planned and natural disinfection processes, and lack of fundamental knowledge concerning their fate and movement through soils. The epidemiological link between viral disease and virus transmission by the water route has been reviewed (Mosely, 1967; Craun and McCabe, 1973; Burge and Marsh, 1978). The ability of viruses to survive most wastewater treatment practices including chlorination, and the numbers and variety of viruses present in treated wastewater applied to land have been discussed by Sagik et al. (1978) and Gerba et al. (1975a), respectively. Gerba (1975b) and Vilker (1980c) have reviewed the qualitative reports on the movement of viruses through percolating beds of soils and soil components.

This final technical report gives the results of a three year research project that is directed toward modeling and performing experiments which will lead to a quantitative description of virus movement in soils. The report is divided into four sections which describe the adsorption transport model and its application, describe experiments using attenuated poliovirus that are used to measure necessary model parameters, present conclusions and on-going activities, and outline future research needs. Appendix I lists the articles and student theses which were published with the assistance of this project grant.

II. THEORY AND ANALYSIS

Virus movement in percolating soil water depends on wastewater application rate and composition, virus type, frequency and amount of rainfall and soil composition. Ultimately it is desired to find a model of this movement which incorporates these variables in terms of easily measured or calculated parameters. The objective is to describe the breakthrough or concentration history of virus in a soil bed effluent. This section describes an initial modeling effort for the case of single virus particle movement through the soil matrix and negligible inactivation relative to the time scale of transmission. In order to obtain initial analytical results for our model several additional simplifications are used: (1) percolate flow applied to the bed of soil or soil components is steady and continuous, (2) applied virus concentration, C_o, is constant with time, 3) bed packing density is homogeneous and, 4) bed is initially saturated with percolate solution containing no virus. These simplifications can be removed by more sophisticated formulations of the basic model along with computer algorithms when further physical details become available. These simplifications are in accord with the manner in which many of the previous laboratory column studies of virus transmission were conducted (Vilker, 1980c).

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Virus Mass Balance

The approach is based on the reversibility of infective virus particle association with soils and soil components. At equilibrium, the extent of this association is usually described by an adsorption isotherm. However, describption of the rate of approach to this equilibrium virus distribution between the solid-adsorbed state and the liquid-supernatant state requires an analysis of adsorption mass transfer. This involves diffusion of virus particles to the solid surface and must include the local hydrodynamic effects of the fluid-phase near the surface. It is important to recognize that in this unsteady-state process, virus concentration at any point in the bed changes with time.

For time t after the start of flow of the percolate containing virus at concentration C_o to the bed, a differential virus mass balance at depth z gives

$$\frac{\partial C}{\partial t} + u \frac{\partial C}{\partial z} + \frac{\rho_B}{\varepsilon} \frac{\partial q}{\partial t} = 0 \qquad (1)$$

where $C(t,z)$ is liquid-phase virus concentration at time t and depth z, $q(t,z)$ is solid-phase virus concentration, ρ_B is mass of solid per unit mixed volume (solid + fluid), ε is bed void fraction and u is pore fluid velocity. Volumetric flow rate applied to the top of the bed is $\varepsilon u A_c$ where A_c is bed cross-sectional area. The rate of adsorption at any z is given by

$$\rho_B \frac{\partial q}{\partial t} = \hat{\kappa}_{f,c}(C-C^*) \qquad (2)$$

where $\hat{\kappa}_{f,c}$ is an adsorption rate parameter which accounts for the diffusive transport of virus from the bulk solution through a quiescent liquid layer adjacent to the surface of each solid particle and C^* is virus concentration in the liquid in immediate contact with this surface. In order to solve equations (1) and (2), initial and boundary conditions must be specified and an isotherm relationship must be available for $q(C^*)$. For the simple case analyzed here, the initial condition is that the bed is saturated with percolate solution which contains no virus so that $C(0,z) = q(0,z) = 0$, and the boundary condition is that after the start of percolation, bed influent virus concentration is constant, $C(t,0) = C_o$.

Adsorption Isotherms and Breakthrough Curves

The choice of analytical expressions to describe $q(C^*)$ and $\hat{\kappa}_{f,c}$ depends on the adsorption isotherm. Virus adsorption to most solids is probably best described in terms of the saturation (or Langmuir) isotherms (Vilker and Burge, 1980b):

$$q = QK_L C_e/(1+K_L C_e) \qquad (3)$$

(continued)

where the adsorbent concentration q (virus/unit mass adsorbent) is in equilibrium with liquid-phase concentration C_e (virus/unit volume solution) as typically measured in a batch experiment. C^* and C_e are both liquid-phase equilibrium concentrations (with q), but the former is conventionally used to designate the interfacial concentration in a packed bed. Q is the maximum number of adsorption sites per unit mass adsorbent and K_L is the equilibrium constant which is a measure of the strength of adsorption. When adsorption is weak (K_L small) or the batch experiment is performed at insufficiently high virus concentrations (C_e small), then $K_L C_e << 1$ and the observed isotherms will appear to be

$$q \simeq (QK_L)C_e \tag{4}$$

In these cases the parameters Q and K_L cannot be uniquely determined. An analysis of many reported batch measurements has shown that the product (QK_L) varies by five orders of magnitude among various virus-solid sorbent combinations, but most measurements were made at levels of C_e which are too small to allow determination of the separate parameters (Wilker and Burge, 1980b). In those cases where a determination was possible, the large values of Q, and the small values of K_L indicate that virus adsorption is characterized by a large number of sites, but that equilibrium greatly favors the liquid-phase over the adsorbed-phase, that is, the adsorption is weak.

This conclusion has important consequences for the solution of equations (1), (2) and (3) to describe virus movement in laboratory packed columns or field applications. This can be shown by examining the breakthrough curves for the limiting cases of strong and weak adsorption as shown in Figure 1. The curves show the soil water virus concentration as a function of time and bed depth, $C(t,z)$. Strong and weak adsorption are quantitatively defined in terms of the separation factor

$$r = 1/(1+K_L C_o) \tag{5}$$

At a given value of the virus concentration in the liquid applied to the bed, C_o, sufficiently large K_L gives $r << 1$ and the adsorption is characterized as strong. In this case, the solutions of Equations (1), (2) and (3) give curves like those of Figure 1A which show virus concentration at early time t_1 to decrease as a sigmoidal function of bed depth from C_o near the inlet where the solid phase is saturated. Once formed, this total concentration profile moves down the bed without distortion as more of the solid-phase becomes saturated. Finally, at some later time t_4, the first virus begins to appear in the bed effluent. A rapid rise in effluent concentration will occur when the bed solid-phase is saturated with virus. When K_L is sufficiently small, $r \sim 1$ and adsorption is weak. In this case breakthrough patterns will resemble those in the schematic of Figure 1B. The time to first appearance of virus in the bed effluent for weak adsorption is much less than in the case of strong adsorption (i.e. t_4, Figure 1B $<< t_4$, Figure 1A), and less of the solid-phase will be saturated when equivalent effluent concentration is reached.

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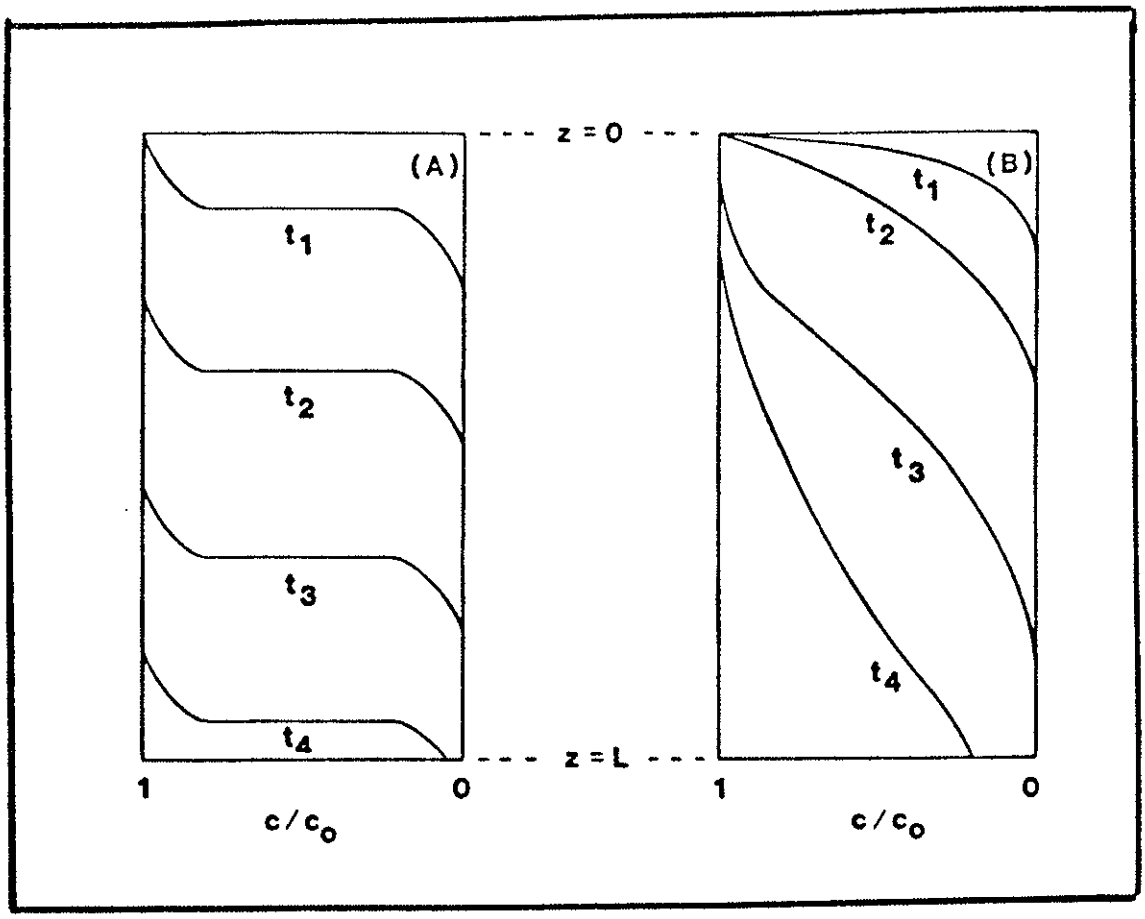

Figure 1. Virus breakthrough curves from packed beds of length L corresponding to limiting cases of (A) strong adsorption, $r \ll 1$ and (B) weak adsorption, $r \sim 1$. Times in (B) are much shorter than equivalent times in (A).

Adsorption Isotherm and Column Breakthrough Curve Analysis for ϕx-174 Bacteriophage Adsorbing to Kranzburg Silt Loam Soil

The previous discussion shows the importance of characterizing the equilibrium adsorption isotherm between viruses and soil components which serve as their adsorbents. Most previously reported isotherm determinations for virus adsorption have been conducted at liquid-phase concentrations much greater than those concentrations used in field applications, but well below levels required to obtain the parameters Q and K_L of Equation (3), thereby preventing evaluation of the separation factor r by Equation (5). Burge and Enkiri (1978) reported one such high virus concentration experiment for the adsorption of ϕx-174 bacteriophage adsorbing to 2 mm particles of Kranzburg silt loam (K sl) soil. The analysis of the data by Equation (3) is shown in Figure 2 to give a good fit of the data (Vilker and Burge, 1980b). This fit gives $Q = 2.07 \times 10^{8}$ sites/mg and $K_L = 4.97 \times 10^{-11}$ ml/virus.

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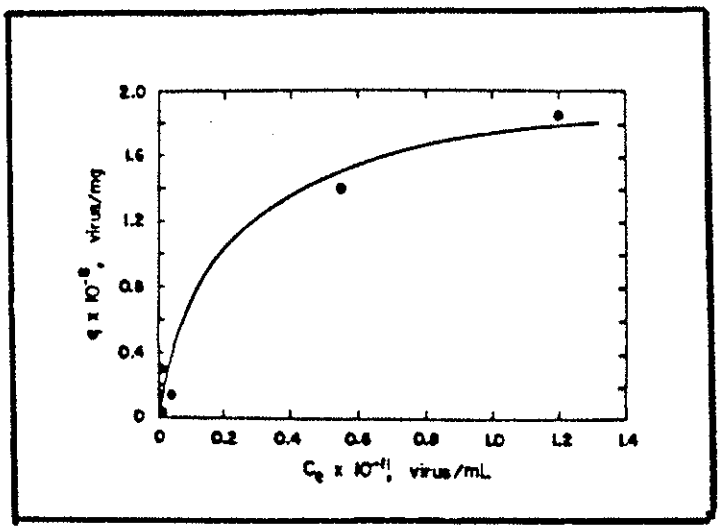

Figure 2. Saturation adsorption of ϕx-174 phage in 0.1 M NaCl to untreated K sl soil. Curve calculated by equation (3) with $Q = 2.07 \times 10^{8}$ sites, mg^{-1} and $K_L = 4.97 \times 10^{-11}$ ml $virus^{-1}$.

For this small value of K_L, $r_8 \approx 1$ will apply to influent ϕx-174 phage concentrations of the order 10^8 virus/ml or less. In this case, Eq. (3) is simplified with $C^* = q/QK_L$ and $\kappa_{f,c} = k_{f,c} a_p$, where $k_{f,c}$ is the liquid-solid interfacial mass-transfer coefficient evaluated for the flow condition in the bed and a_p is outer surface interfacial area of solid sorbent per unit volume of contacting system (solid + fluid). The analytical solution of Eqs. (1) and (2) give the liquid - and solid-phase virus concentrations as functions of time and bed depth,

$$\frac{C}{C_o} = J(N,NT) \tag{6}$$

$$\frac{q}{q_o} = 1 - J(NT,N) \tag{7}$$

where

$$N = \frac{\hat{\kappa}_{f,c} z}{\varepsilon u} \tag{8}$$

$$T = \frac{\varepsilon(ut - z)}{(QK_L)\rho_B z} \tag{9}$$

$$J(\alpha, \beta) = 1 - e^{-\beta} \int_o^{\infty} e^{-\xi} I_o\left(2\sqrt{\beta \xi}\right) d\xi \tag{10}$$

(continued)

Dimensionless groups N and T are known as the column-capacity and throughput parameters, respectively. These analytical expressions are solved in Vilker and Burge (1980b) for the case of percolation of a solution containing 10^8 ϕx-174 virus/ml or less through a 100 cm column packed with 2 mm particles of K sl soil. The results are shown in Figure 3.

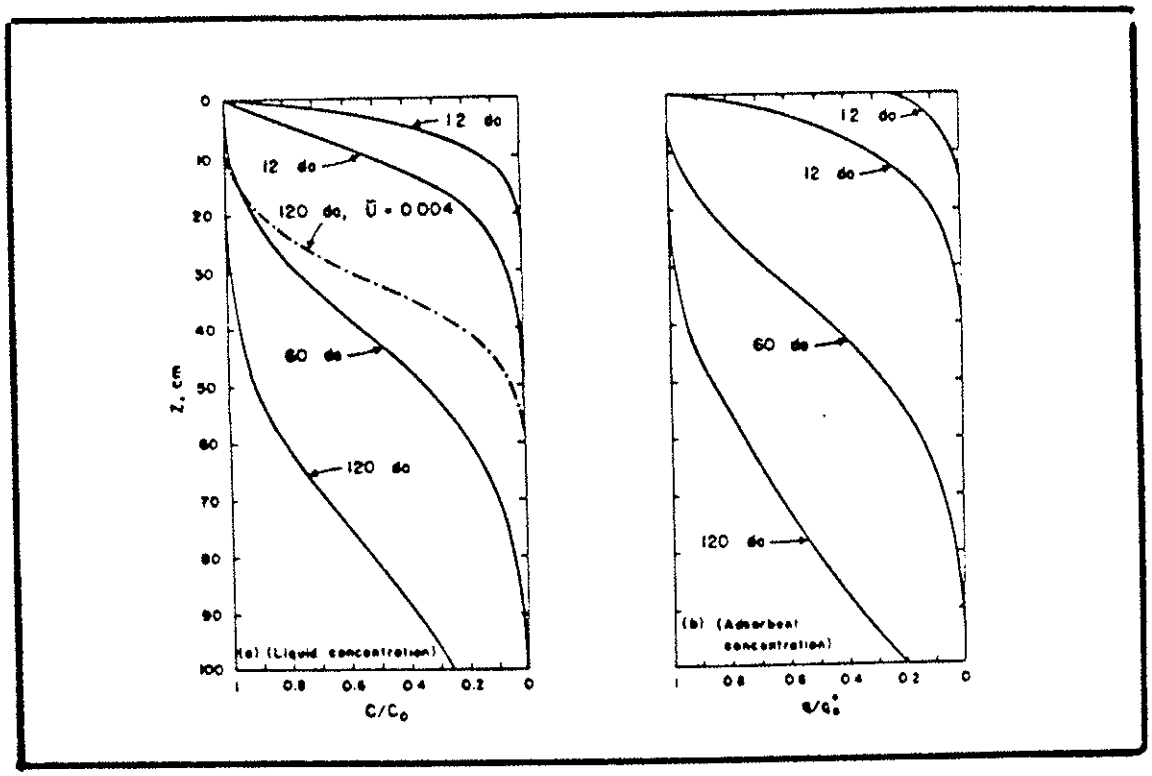

Figure 3. (a) Calculated liquid-phase virus profiles for ϕx-174 phage adsorption to K sl soil at percolation rate $\bar{U}$ = 0.01 cm/min (———), and at $\bar{U}$ = 0.004 cm min^{-1} (—·—). (b) Calculated adsorbent-phase phage profiles for K sl soil at $\bar{U}$ = 0.01 cm/min.

The solid curves in the figure show virus profiles as functions of time and depth at percolation rate of $\bar{U}$ = 0.01 cm/min which corresponds to an application rate of about 40 in/wk. After 1.2 days, virus has penetrated to about 25 cm depth and after about 60 days the first virus begins to appear in the effluent of a one meter column. After 120 days the effluent concentration is about 25% of influent. The dashed curve of Fig. 3a shows the increased retention of virus at 120 days which results when percolation rate is reduced by 60%. Figure 3b shows the fractional saturation of the soil as a function of time and depth. At 120 days, the top 20 cm of soil contains the maximum amount of virus it can retain when in contact with a liquid at virus concentration C_o.

From Fig. 3 it is clear clear that the timescale of virus transport through the one meter bed is sufficiently long that natural inactivation would affect the interpretation of virus breakthrough. While the total

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virus profiles shown in Fig. 3 may or may not be altered due to inactivation, the fraction of infective viruses at a given depth would probably be significantly reduced from the values shown, dependent on adsorption column temperature.

III. EXPERIMENTS WITH POLIOVIRUS

This section addresses the need for experimental data required to apply the model described above. Attenuated poliovirus was selected on the bases that it is an enteric virus, and therefore representative of the class of viruses that is found in sewage; it is safe to work with; its concentration can readily be determined by plaque assay with primary tissue culture cells from commercial sources; and it could be easily obtained from the UCLA Department of Virology. Experiments were performed to test the hypothesis that single virus particles could be present in treated wastewater, to determine the adsorption isotherm for single poliovirus particles adsorbing to a well-characterized size fraction of Ottawa sand, and to study virus interactions with montmorillonite clay.

Poliovirus Association with Wastewater Treatment Solids

The relative numbers of free, aggregated, adsorbed and embedded virus particles in raw and treated wastewater is poorly understood. Wellings et al. (1976) have shown that 16 to 100% of the total virus demonstrated in samples of raw sewage and chlorinated effluent was solids associated. The capacity of activated sludge solids to adsorb coxsackie virus and stabilization pond solids to adsorb poliovirus have been studied by Clarke et al. (1961) and Sobsey and Cooper (1973), respectively. Results of both studies were expressed in terms of isotherms of the form $q = uC_e^n$, where q is the number of virus particles adsorbed per unit mass of solids, C_e is virus concentration in the liquid phase, and u and n are constants evaluated for a single concentration of suspended solids. The studies also showed that equilibrium of virus with the solids was rapid, that desorbed virus retains its infectivity and, that the capacity of the suspended solids decreased with increasing solids concentration. We undertook a study of adsorption of poliovirus to suspended solids from an activated sludge unit in order to define adsorption capacity over a wider range of solids concentration than has been previously reported (Vilker et al., 1980a). Adsorption was found to be linearly dependent on liquid-phase virus concentration (i.e. $n = 1$) but inversely dependent on solids concentration. In order to account for this solids concentration dependence, the results are correlated by the isotherm relationship

$$q = \frac{C_o - C_e}{w} = v w^{p-1} C_e \qquad (11)$$

where $(C_o - C_e)$ is the number of virus particles per mL adsorbed by the mass of suspended solids, w mg/mL, and the coefficients determined by linear regression of the data are $v = 0.63$ and $p = 0.193$. This equation can be rearranged in order to show the effect of increasing solids concentration on the ratio of free unassociated to total virus in the suspension medium:

$$\frac{C_e}{C_o} = \frac{1}{1+uw} \qquad (12)$$

(continued)

where u is constant in the earlier studies but a function of solids concentration in our work. Figure 4 shows this ratio as a function of solids concentration. At high solids concentrations typical for an activated sludge mixed liquor (5 mg/mL), more virus remain in the liquid phase than would be predicted from earlier measurements while at low solids concentration typical for treated wastewater effluent, 80% of total virus remain unadsorbed as opposed to 99% from the earlier studies.

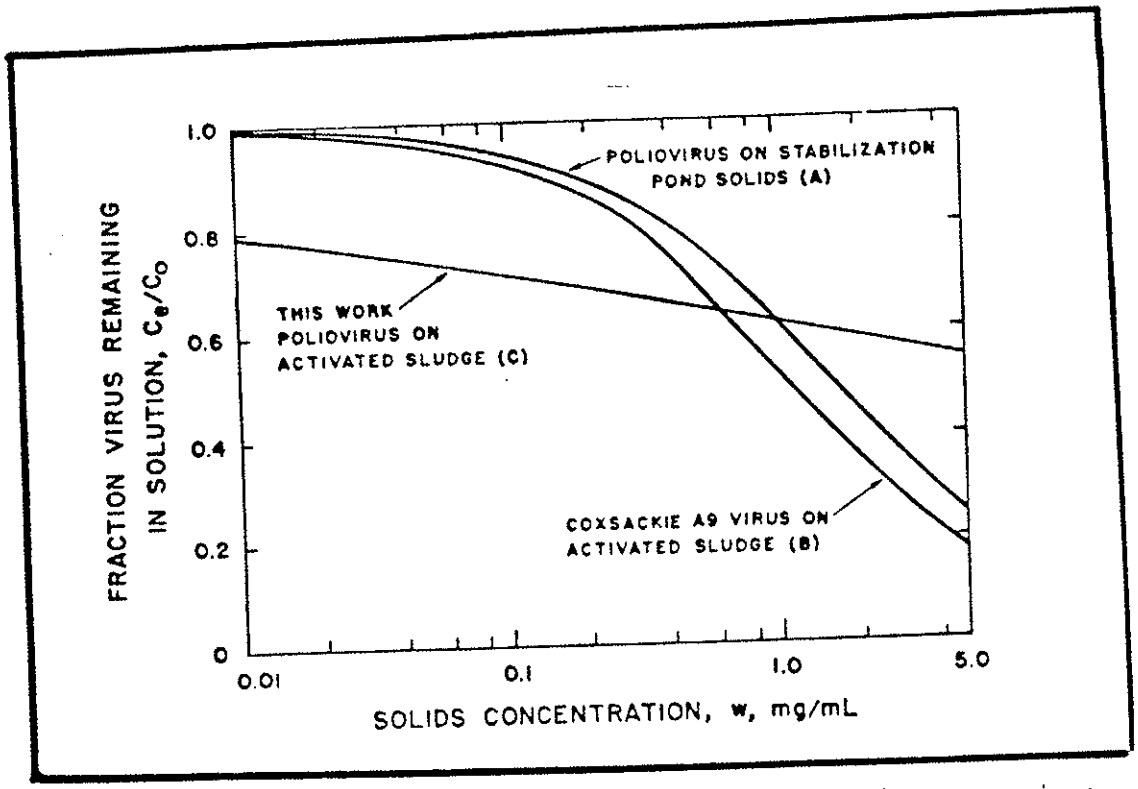

Figure 4. Fraction of virus remaining unadsorbed in suspensions of biotreatment solids. In Eq. (12), $u = 0.63$ for curve (A); $u = 0.91$ for curve (B); $u = 0.63\ w^{-0.81}$ for curve (C). (Vilker et al., 1980a).

We concluded from these distribution studies that a high percentage of those virus particles which survive disinfection, or are released from the embedded matrix after disinfection are released to the environment in the unassociated state. This gives us confidence in the applicability of the adsorption model to the description of virus movement in soils.

Poliovirus Adsorption to Ottawa Sand

A narrow particle -size fraction of Ottawa sand was prepared by combining dry and wet sieving methods. The particle-size distribution of this fraction was 123 ± 24 μm (Feret's diameter) as determined with an optical microscope (Fong, 1980).

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High titer attenuated poliovirus I preparations (10^9-10^{10} virus/mL) were made by growing the virus on African Green Monkey Kidney cells followed by concentration and purification using density gradient ultracentrifugation. Details of the methods and the plaque-forming assay method for determining infective virus concentration are available elsewhere (Fong, 1980). Adsorption measurements were made by equilibrating solutions of virus and sand suspended in phosphate buffered saline (100 mg sand/L solution, ~0.17 M ionic strength, 7.2 pH) at 20°C with gentle agitation.

The adsorption measurements were fit by the saturation isotherm function, Equation (3), with the coefficients $K_L = 3.60 \times 10^{-9}$ mL/virus and $Q = 3.56 \times 10^{8}$ virus sites/mg determined by nonlinear least squares regression. From this value of Q and the sand particle size it is estimated that adsorption sites occupy about 1.5% of the total sand particle surface area. The low value for the equilibrium constant, K_L, indicates that the adsorption reaction of polio to sand is also weak.

Poliovirus Adsorption to Montmorillonite Clay

Our first attempts to measure the poliovirus adsorption isotherm for montmorillonite clay gave results of unsatisfactory reproducibility. The poliovirus preparations and the batch adsorption suspensions were of the same composition as used in the sand adsorption studies (ionic strength 0.17 M, pH 7.2). Clay particle suspensions were prepared by dispersing montmorillonite clay powder (Ward's Natural Science Establishment, Inc., Rochester, NY, #46W 0435 PAC) in phosphate buffered saline and decanting the suspension after large agglomerates were separated by gravity settling.

Preliminary studies to find the causes of the large data scatter included varying the concentration of clay particles in the adsorption suspensions by dilution methods and attempting to control clay particle size. Adsorption was found to decrease slightly as clay concentration increased over the range of 10^{-4} to 1 mg clay/mL solution, but the effect was much smaller than the variability of the data. Nominal clay particle size was varied by increasing the time of settling during gravity sedimentation as suggested by Tanner and Jackson (1947): 1 hr settling gives 6 µm particles, 4 hrs settling gives 3 µm particles, 15 hrs settling gives 1.5 µm particles. The measurements failed to show a recognizable trend to changes in this nominal particle size parameter.

An isotherm was derived by least squares regression using the function of Equation (4) for which $(QK_L) = 200$ mL/mg. This value of the product of the isotherm parameters is more than 100 times larger than the value for adsorption to sand (Meronek, 1978).

We concluded that further analysis of poliovirus adsorption to clay would require better understanding of the particle interactions in the clay-virus-electrolyte suspensions. In particular, we desired to know more accurately the true size of the clay particles and the interactions between individual clay and virus particles. Clay suspensions obtained from gravity settling of dispersions of the powder in distilled water were chemically treated and further fractionated to achieve a monodisperse, narrow particle-size distribution. The chemical treatments for dissolving carbonates, removing soluble ions, residual organic material and iron oxides described by

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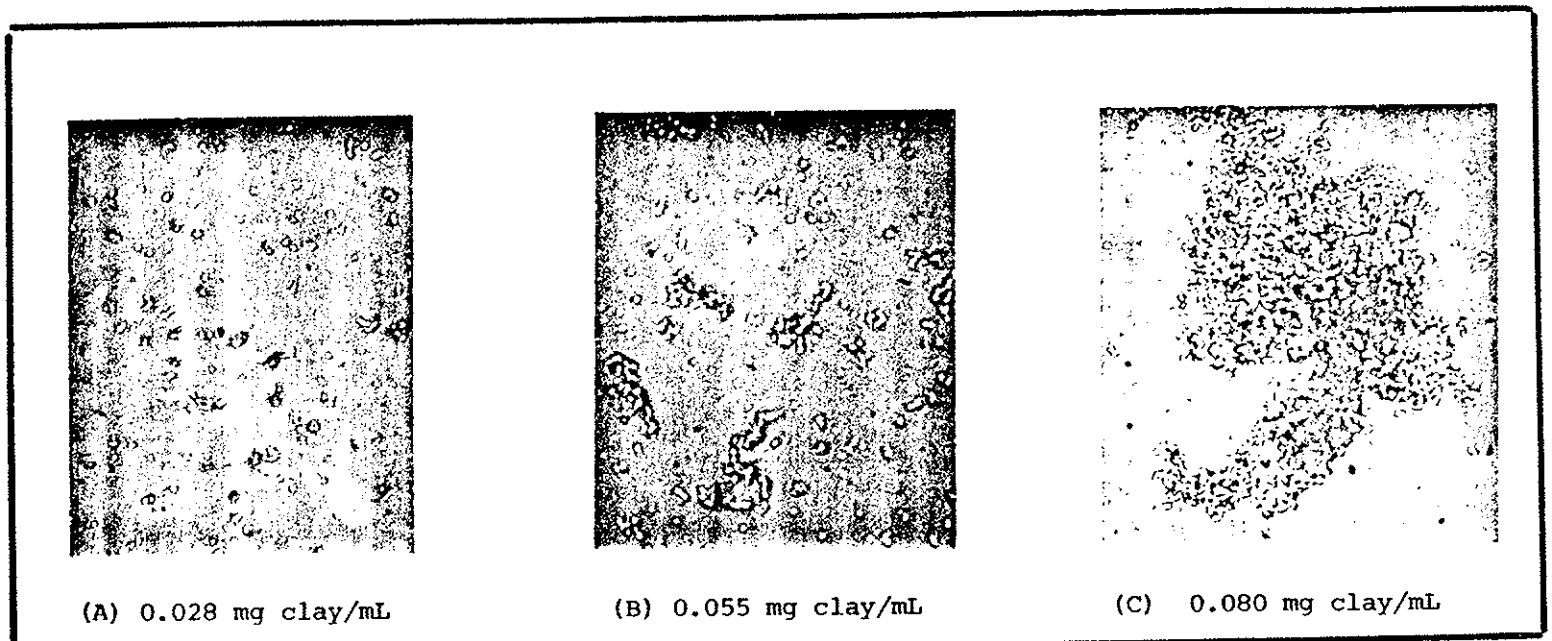
Jackson (1965) were used. Particle size fractionation was done by centrifugal separation methods. Monodisperse preparations of particles with a mean equivalent spherical diameter of 1-2 μm were obtained by these techniques with size measurement done using a calibrated eye-piece micrometer on a Zeiss Model 20T microscope (640X total magnification) (Butler, 1980).

The sequence of photomicrographs in Figure 5 shows that essentially single 1-2 μm clay particles exist for virus adsorption at low clay concentrations but that clay aggregation takes place as clay concentration is increased. In the absence of virus, no clay aggregates were observed at much higher clay concentrations (0.69 mg/mL) than the concentration of Figure 5c. We have also observed that clay aggregation is sensitive to electrolyte ionic strength and virus concentration but systematic studies of these variables have not been completed. Formation of clay aggregates such as the one in Figure 5c are expected to influence adsorption isotherm determinations. We are proceeding to determine the isotherm at conditions of clay concentration and ionic strength where aggregate formation is minimized.

These optical and additional scanning electron microscopy studies reported by Butler (1980) give reasonable assurance that poliovirus is removed from the clay suspensions of batch equilibrium experiments by the adsorption mechanism. This is also probably the principal mechanism of removal from solutions percolating through packed beds in which clay particles are not likely to move together to form aggregates. We now are proceeding to improve our determination of the adsorption isotherm by assuring that clay aggregation effects are absent or minimized. These effects could interfere with a true adsorption mechanism by enhancing virus removal by the sweep-floc mechanism, or decrease removal by decreasing the number of clay edges due to their involvement in edge-to-surface clay-clay interactions.



(A) 0.028 mg clay/mL (B) 0.055 mg clay/mL (C) 0.080 mg clay/mL

Figure 5. Photomicrographs (640X) of 1-2 µm montmorillonite clay particles suspended in solutions of phosphate buffered saline (0.026 M, pH 7) and poliovirus ($8x10^7$ virus/mL) showing increasing flocculation with increasing clay concentration. (Butler, 1980)

Conclusions from Adsorption Isotherm Studies

Adsorption isotherm determinations for the virus-solid combinations discussed earlier are summarized in Figure 6. The isotherm for T_4 bacteriophage adsorption to activated carbon by Cookson and North (1967) has been added since it is the only other example found in the literature for which adsorbent particle size was accurately known and measurements were made at sufficiently high C_e to show the saturation effect.

The table which follows summarizes the adsorption parameters determined for these isotherms. In the case of our incomplete study of poliovirus adsorption to clay we have assumed the order of magnitude for the number of adsorption sites/mg clay to be 10^9 in order to arrive at an estimate for K_L. These data show that for all virus/adsorbent combinations, adsorption can be classified as weak (small K_L) but that the relative strength of adsorption ranges over about three order of magnitude.

Since the ϕx-174/silt loam combination displays the "weakest" adsorption strength of the data summarized in the table, we would expect the breakthrough curves for the other virus/adsorbent pairs to be of similar shape as the curves shown in Figure 3 but correspond to longer times. That is, T_4-phage liquid-phase concentration profile in an activated carbon bed would probably be about the same shape as the ϕx-174/silt loam profile for breakthrough at a comparable percolation rate, but time to T_4 breakthrough would be much longer than 60 days.

IV. CONCLUSIONS AND ON-GOING RESEARCH

Virus adsorption to activated carbon, soils and soil components is saturation limited. Although these materials have large capacity, the association of virus particles with them is characterized as weak due to the small value of the isotherm equilibrium parameter K_L. This parameter can be determined for specific virus/adsorbent combinations from batch solution experiments with measurements extended to high virus concentrations. This method cannot be used for adsorbent materials such as finely divided montmorillonite clay due to aggregation of adsorbent particles at high virus concentrations.

Knowledge of adsorption equilibrium and rate phenomena can be used in an adsorption mass transfer model to predict single virus particle breakthrough from percolating soil columns. The model was applied to show that ϕx-174 phage breakthrough from one meter long columns of silt loam soil would occur after about 60 days when percolation rate was 40 in/wk. The breakthrough of poliovirus from one meter of sandy soils at equivalent percolation rates is expected to be much longer based on the 50-fold higher value of the adsorption equilibrium parameter (QK_L) for the polio/sand combination relative to the ϕx-174/K sl soil combination.

Current follow-up research, supported in part by renewal grant W-597 from the California Water Resources Center, is directed toward experimental verification of the adsorption mass transfer model for poliovirus breakthrough from columns packed with the same size-fraction of Ottawa sand

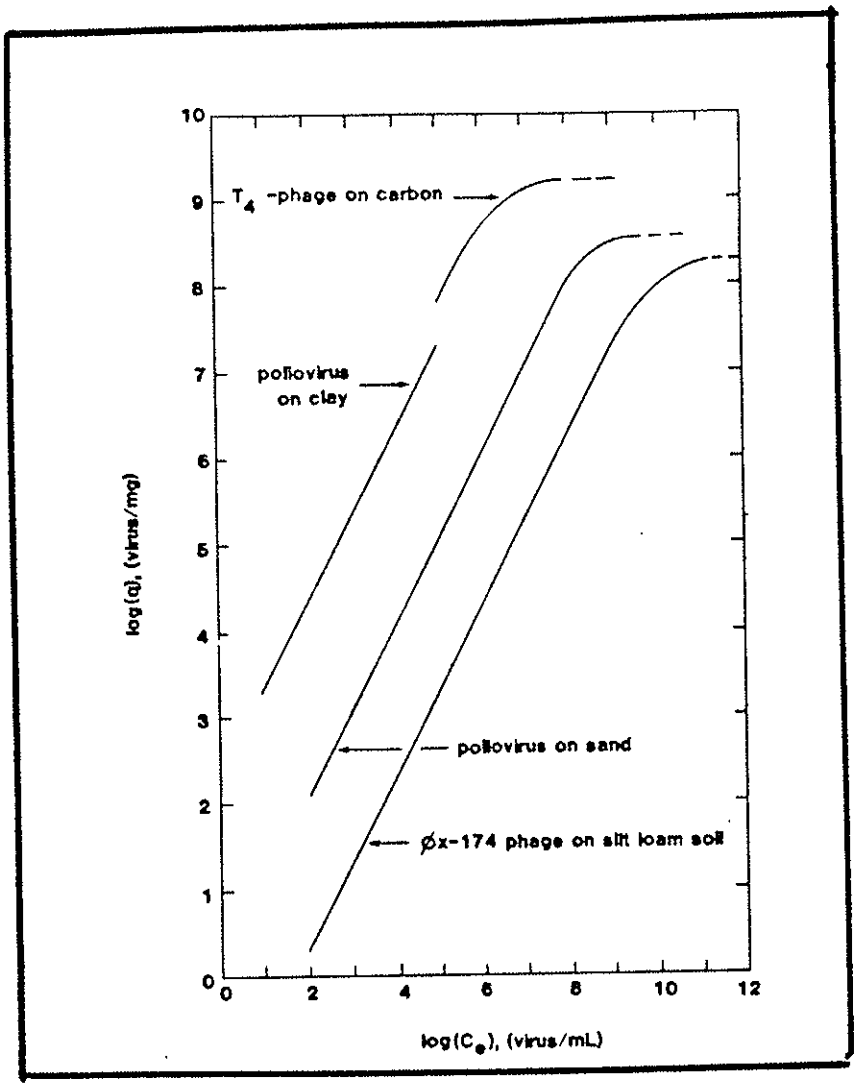


Figure 6 . Adsorption isotherms for T_4-bacteriophage on activated charcoal particles, d = 2.5 ± 2.0 μm (Cookson and North, 1967); poliovirus on montmorillonite clay particles, size unknown (Meronek, 1978); poliovirus on Ottawa sand particles, d = 123 ± 24 μm (Fong, 1980); ϕx-174 bacteriophage on silt loam soil, d = 2 mm (Vilker and Burge, 1980).

Virus/Adsorbent	QK_L mL/mg	Q sites/mg	K_L mL/virus
T_4-phage/carbon	640	1.6×10^9	4.00×10^{-7}
Polio/clay	200	10^9 (assumed)	2.00×10^{-7}
Polio/sand	1.28	3.56×10^8	3.60×10^{-9}
ϕx-174/silt loam soil	0.02	1.89×10^8	1.05×10^{-10}

for which the isotherm data described above has been obtained. We are also continuing the study of poliovirus interactions with 1-2 μm montmorillomite clay particles. Recent high-precision adsorption isotherm measurements at 22°C and low virus concentrations where clay aggregation effects are absent will be coupled with similar measurements at 1°C in order to obtain necessary adsorption equilibrium parameters Q and K_L.

V. FUTURE RESEARCH NEEDS

In this section, we briefly outline research needs which extend beyond this completed project W-523 or the one year renewal project W-597, but would provide valuable fundamental understanding to ensure protection of fresh water supplies from viral contamination.

Most viruses that enter sewage-treatment plants are associated with solid particles, either embedded within the particles or adsorbed to surfaces. The fate of viruses that survive treatment, including disinfection, depends on the extent of this association at point of application to soil. Virus distribution among the range of suspended particle sizes needs to be known in order to establish the limiting mechanism of removal by soils.

Although it is likely that single virus particle movement is important during land-treatment applications, it is unlikely that this movement will ever be described completely by modeling. However, the use of modeling along with laboratory observations can provide a method for ranking soils according to their relative strength to detain, and to permanently inactivate enteric viruses. To accomplish this, more experimental measurements of the sorptive capacity of several soils, and soil components, for different enteric viruses are required. Better understanding needs to be developed about the sorptive interaction in nearly salt-free waters, such as rainwater. Currently, no quantitative measurements of sorptive capacity in these waters are available. Modeling efforts need to be extended to assess the extent and rate of virus elution from soils.

Complete description of virus movement must integrate sorptive interactions and permanent inactivation. Experimental techniques need to be developed for determining the fraction of eluted virus which remains infective. Better understanding of the differences in inactivation rates for viruses freely suspended in soil water and viruses adsorbed to soil particles will help determine the best management of drying land-treatment sites for short periods between wastewater applications. The relationship between soil virucidal activity and chemical and microbiological composition needs to be better understood.

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