

# Evaluation of the Factors Controlling the Time-Dependent Inactivation Rate Coefficients of Bacteriophage MS2 and PRD1

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Static and dynamic batch experiments were conducted to study the effects of temperature and the presence of sand on the inactivation of bacteriophage MS2 and PRD1. The experimental data suggested that the inactivation process can be satisfactorily represented by a pseudo-first-order expression with time-dependent rate coefficients. The time-dependent rate coefficients were used to determine pertinent thermodynamic properties required for the analysis of the molecular processes involved in the inactivation of each bacteriophage. A combination of high temperature and the presence of sand appears to produce the greatest disruption to the surrounding protein coat of MS2. However, the lower activation energies for PRD1 indicate a weaker dependence of the inactivation rate on temperature. Instead, the presence of air–liquid and air–solid interfaces appears to produce the greatest damage to specific viral components that are related to infection. These results indicate the importance of using thermodynamic parameters based on the time-dependent inactivation model to better predict the inactivation of viruses in groundwater.

## Introduction

Sources of pathogenic viruses to drinking water supplies include septic tanks, broken sewer lines, improperly constructed landfills, open dumps, or intentional groundwater recharge and crop irrigation with treated municipal wastewater (1, 2). In all these cases, viruses released into the subsurface environment may remain infective and survive for a considerable period of time (i.e., weeks to months). As a consequence, these microorganisms can infiltrate through the unsaturated zone and, upon reaching the water table, can continue to migrate downstream to points of withdrawal.

The most important processes controlling the fate and transport of viruses in the subsurface are sorption and inactivation (3, 4). Virus sorption is affected by several factors including viral surface properties, groundwater quality, and soil surface charges (5). The process of virus inactivation can result from alteration of the surrounding protein coat

contained in the viral capsid. Early studies determined that the inactivation of viruses in groundwater appears to be influenced by a number of factors including virus type, soil characteristics, groundwater quality, and the presence of microorganisms (6–9). However, published results on the significance of some of these factors on virus inactivation are not consistent. The only factor found to consistently control the inactivation of viruses in groundwater is temperature (10). Viruses and viral nucleic acids are presumably inactivated by a first-order process, and the reaction is strictly dependent on temperature (i.e., the reaction appears to be monomolecular) (11). However, various attempts to predict virus inactivation in groundwater as a function of temperature by using constant inactivation rate coefficients have been of limited success (12, 13). One possible explanation for these differences might be the presence of two (biphasic) or more (multiphasic) viral subpopulations undergoing sequential inactivation with different inactivation rate coefficients (14–17). Therefore, accurate prediction of virus inactivation should account for temporal variation of the inactivation rate coefficients.

In the present investigation, the inactivation process is represented by a pseudo-first-order expression with a time-dependent rate coefficient. Static and dynamic batch experiments were performed to compare the effects of temperature and the presence of sand on the time-dependent rate coefficient. Finally, the ability to more accurately predict the inactivation of viruses in groundwater by using thermodynamic parameters obtained from time-dependent inactivation rate coefficients is discussed.

## Materials and Methods

**Bacteriophage Assays.** The single-stranded RNA male-specific coliphage, MS2, and the double-stranded DNA somatic *Salmonella typhimurium* phage, PRD1, were used as model viruses in this study. These bacteriophages have been used extensively in virus inactivation studies and are considered to be good model viruses because they behave more conservatively (lower sorption) than many pathogenic viruses and are capable of surviving for significant periods of time in groundwater (18–21). Bacteriophage concentrations were measured by using the double-agar-layer assay method (22). PRD1 bacteriophage was analyzed by using *Salmonella typhimurium* LT2 (ATCC no. 15277) as the host bacterium. For the analysis of MS2 bacteriophage, *E. coli* HS( $\rho$ F<sub>amp</sub>)R (ATCC no. 700891) were used as the host bacterium with the same concentration of antibiotics, as described by Debartolomeis and Cabelli (23).

The double-agar-layer assay method was performed by preparing a mixture of 1.0 mL of host bacteria, 4.0 mL of molten soft agar (50 °C), and 1.0 mL of sample. To ensure that all host bacteria were in log-phase growth, 0.1 mL of prepared stocks of each host were transferred to a test tube containing 10 mL of trypticase soy broth (Difco) and placed in a shaker at 37 °C for 4 h or until cultures were visibly turbid. The mixture was gently vortexed and poured onto the appropriate plating medium. Plates were incubated at 37 °C for 24 h. The concentrations were determined by counting the number of plaques on each plate. Plates were made in duplicate, and the concentration was averaged from the plaques counted on each plate. The double-agar-layer assay used for this study was estimated to have a detection limit of 0.5 plaque-forming units (PFU)/mL.

**Static and Dynamic Batch Experiments.** A low-ionic-strength phosphate buffered saline (PBS) solution was prepared with 0.25 mM Na<sub>2</sub>HPO<sub>4</sub>, 1.2 mM NaCl, and 0.037

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mM KCl in UV-disinfected distilled water with a specific conductance of 17.8  $\mu\text{S}/\text{cm}$  and adjusted to a pH of 7.5 with HCl. The resulting specific conductance of the pH-adjusted PBS solution was about 200  $\mu\text{S}/\text{cm}$  and corresponds to an ionic strength of 2 mM. Prepared stocks of bacteriophage MS2 and PRD1, with concentrations in excess of  $10^8$  PFU/mL, were diluted with the PBS solution to yield bacteriophage concentrations of about  $5.0 \times 10^5$  and  $5.5 \times 10^5$  PFU/mL, respectively.

Over 100 oven-baked 40-mL borosilicate glass bottles were filled with 10 mL of the pH-adjusted PBS solution containing bacteriophage MS2 and PRD1. Half of the bottles were filled with 10 g of no. 60-sieved, presoaked, oven-dried Monterey sand (RMC Industries, Decatur, GA) and gently vortexed to ensure that the sand was completely wetted by the PBS solution.

The bottles were arranged into four groups (three static and one dynamic) with two sets of bottles in each group. Each set consisted of 14 bottles containing virus suspensions with sand and 14 bottles containing virus suspensions without sand. For the static batch experiments, one group of bottles was placed in a refrigerator at 4 °C, one group was placed in a constant-temperature room at 15 °C, and one group was placed in an incubator at 25 °C. The dynamic batch experiment was performed with all the bottles attached to a small benchtop tube rotator (Glas-Col, Terre Haute, IN) placed in the constant-temperature room at 15 °C. The bottles were rotated end-over-end, allowing the sand to mix within the PBS solution containing each bacteriophage. At selected times during the experiment, one bottle of each set was chosen at random. Then 1.0 mL of the PBS solution was removed and then assayed for bacteriophage MS2 and PRD1 by using the double-agar-layer assay method. Subsequently, the used bottles were discarded.

## Theoretical Calculations

**Time-Dependent Inactivation.** Sim and Chrysikopoulos (24) and Chrysikopoulos and Vogler (25) have shown that experimental data from numerous batch inactivation studies can be described by a pseudo-first-order expression with a time-dependent rate coefficient as follows:

$$\frac{dC(t)}{dt} = -\lambda(t)C(t) \quad (1)$$

where  $C$  is the concentration of suspended viruses in the liquid phase,  $t$  is time, and  $\lambda_0$  is the time-dependent inactivation rate coefficient of suspended viruses given by:

$$\lambda(t) = \lambda_0 e^{-\alpha t} \quad (2)$$

where  $\lambda_0$  is the initial inactivation rate coefficient, and  $\alpha$  is the resistivity coefficient. Substituting eq 2 into eq 1 and solving the resulting expression subject to the initial condition  $C(0) = C_0$ , where  $C_0$  is the initial virus concentration, yields

$$\ln\left[\frac{C(t)}{C_0}\right] = \frac{\lambda_0}{\alpha}[\exp(-\alpha t) - 1] \quad (3)$$

For the limiting case where the inactivation rate coefficient is considered constant (i.e.,  $\lambda(t) = \lambda$ ), the preceding equation reduces to the form (1):

$$\ln\left[\frac{C(t)}{C_0}\right] = -\lambda t \quad (4)$$

It should be noted that the parameters  $\lambda_0$  and  $\alpha$  can be obtained by fitting eq 3, and  $\lambda$  by fitting eq 4, to the data collected from the static or dynamic batch virus inactivation experiments.

**Thermodynamic Parameters.** The initial inactivation rate coefficient, which depends on temperature and the presence of sand, can be expressed by the empirically derived Arrhenius equation:

$$\lambda_0 = A \exp\left[\frac{-E_a}{RT}\right] \quad (5)$$

where  $E_a$  is the Arrhenius energy of activation,  $A$  is the preexponential factor,  $R = 8.314 \text{ J/mol}\cdot\text{K}$  is the universal gas constant, and  $T$  is temperature in Kelvin. This approach relies on the concept that a molecule must acquire, through random collisions with other molecules, a certain amount of energy,  $E_a$ , before it can undergo a reaction (9). Consequently, the activation energy of the inactivation process can be defined as (26):

$$E_a = -\Omega R \quad (6)$$

where  $\Omega$  is the slope of the plot of  $\ln \lambda_0$  versus  $1/T$  (Arrhenius plot).

By following basic reaction rate theory, the effects of temperature and the presence of sand on the virus inactivation process can be determined by assuming that a thermodynamic equilibrium exists between the concentration of infective viruses and viruses in an activated (transition) state as follows:



where  $C$  is the concentration of suspended viruses in an activated (transition) state and  $C^\ddagger$  is the concentration of noninfective viruses. The equilibrium of suspended viruses in the liquid phase with suspended viruses in the activated (transition) state is defined by a pseudoequilibrium (or "steady-state") constant,  $K^*$ , given as:

$$K^* = \frac{C^*}{C} \quad (8)$$

Furthermore, the first-order expression for the formation of suspended viruses in the activated state can be described by

$$\frac{dC(t)}{dt} = -kC^* \quad (9)$$

where  $k$  is the reaction rate coefficient of the overall inactivation process, which is given by the following relationship:

$$k = \frac{k_B T}{h} \quad (10)$$

where  $k_B = 1.381 \times 10^{-23} \text{ J/K}$  is the Boltzmann constant, and  $h = 6.626 \times 10^{-34} \text{ J/sec}$  is Planck's constant (27). In view of eqs 1 and 9, the time-dependent rate coefficient can be expressed as:

$$\lambda(t) = kK^* \quad (11)$$

Although  $K^*$  cannot be measured directly, it can be related to other standard thermodynamic parameters as follows (11):

$$\Delta G^\ddagger = -RT \ln K^* = \Delta H^\ddagger - T\Delta S^\ddagger \quad (12)$$

where  $G^\ddagger$  is the free energy of formation of the activated (transition) state,  $H^\ddagger$  is the standard enthalpy of formation of the activated (transition) state, and  $S^\ddagger$  is the standard entropy of formation of the activated (transition) state. In view of eqs 2 and 10–12, the value of the thermodynamic parameter  $G^\ddagger$  required to undergo the inactivation process

**TABLE 1. Fitted Inactivation Parameters for Bacteriophage MS2 and PRD1**

conditions	$\lambda_0$ (day <sup>-1</sup> )	$\alpha$ (day <sup>-1</sup> )	$\lambda$ (day <sup>-1</sup> )
<b>MS2</b>			
<b>Static Batch Experiments</b>			
4 °C, sand	$1.5 \times 10^{-2}$	$2.5 \times 10^{-5}$	$2.0 \times 10^{-2}$
4 °C, no sand	$2.6 \times 10^{-2}$	$3.3 \times 10^{-6}$	$2.7 \times 10^{-2}$
15 °C, sand	$6.6 \times 10^{-2}$	$3.4 \times 10^{-6}$	$7.6 \times 10^{-2}$
15 °C, no sand	$6.0 \times 10^{-2}$	$6.7 \times 10^{-6}$	$6.7 \times 10^{-2}$
25 °C, sand	$2.6 \times 10^{-1}$	$4.3 \times 10^{-5}$	$3.0 \times 10^{-1}$
25 °C, no sand	$1.2 \times 10^{-1}$	$9.2 \times 10^{-3}$	$7.9 \times 10^{-2}$
<b>Dynamic Batch Experiments</b>			
15 °C, sand	$7.1 \times 10^{-2}$	$4.2 \times 10^{-3}$	$7.2 \times 10^{-2}$
15 °C, no sand	$9.1 \times 10^{-2}$	$6.7 \times 10^{-5}$	$9.5 \times 10^{-2}$
<b>PRD1</b>			
<b>Static Batch Experiments</b>			
4 °C, sand	$3.1 \times 10^{-3}$	$5.3 \times 10^{-4}$	$4.0 \times 10^{-3}$
4 °C, no sand	$6.7 \times 10^{-3}$	$1.9 \times 10^{-4}$	$7.7 \times 10^{-3}$
15 °C, sand	$2.0 \times 10^{-3}$	$3.0 \times 10^{-4}$	$1.9 \times 10^{-3}$
15 °C, no sand	$2.1 \times 10^{-3}$	$3.5 \times 10^{-5}$	$5.2 \times 10^{-3}$
25 °C, sand	$9.1 \times 10^{-3}$	$6.1 \times 10^{-5}$	$1.0 \times 10^{-2}$
25 °C, no sand	$3.3 \times 10^{-2}$	$1.8 \times 10^{-2}$	$1.4 \times 10^{-2}$
<b>Dynamic Batch Experiments</b>			
15 °C, sand	$1.2 \times 10^{-1}$	$1.5 \times 10^{-2}$	$5.8 \times 10^{-2}$
15 °C, no sand	$1.1 \times 10^{-4}$	$1.2 \times 10^{-2}$	$1.9 \times 10^{-3}$

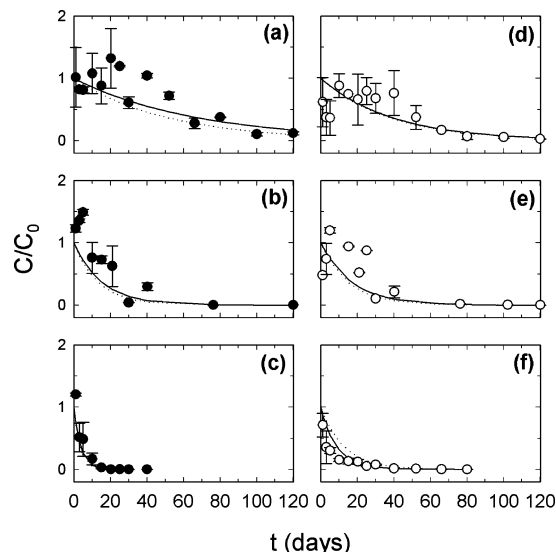
can be expressed as follows:

$$\Delta G^* = -RT \ln \left[ \frac{\lambda_0 h}{k_B T} \right] \quad (13)$$

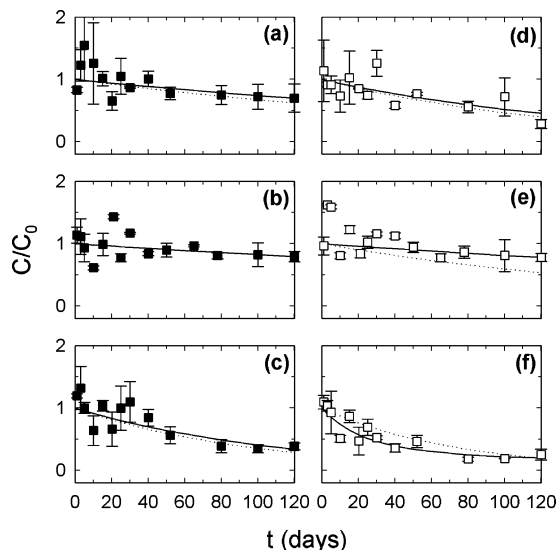
## Results and Discussion

**Model Simulations.** The parameters  $\lambda_0$  and  $\alpha$  were determined by fitting eq 3 to the observed normalized bacteriophage log-concentrations, whereas  $\lambda$  values were determined by linear regression fit to eq 4 of the same experimental data. Note that eq 3 represents time-dependent inactivation, whereas eq 4 assumes that the inactivation rate is constant. Table 1 shows the  $\lambda_0$ ,  $\alpha$ , and  $\lambda$  values for both MS2 and PRD1 with and without sand at 4, 15, and 25 °C. It should be noted that sand containing only trace amounts of ferric oxyhydroxide coatings or organic carbon was used in this study in order to avoid virus adsorption onto the sand grains (28). Furthermore, the low ionic strength of the final PBS solution, along with the low bacteriophage concentrations in the virus suspensions, minimizes any possible loss of virus infectivity due to Brownian coagulation (29–31).

The MS2 concentrations from the static batch experiments with and without sand at temperatures of 4, 15, and 25 °C are shown in Figure 1. The plots indicate that MS2 inactivation is affected by the ambient temperature. It should be noted that normalized bacteriophage concentrations over 1 are due to slight variations in the initial concentration of the virus suspensions present in each bottle. Also presented in Figure 1 are the corresponding simulated concentration histories for both time-dependent and constant inactivation rates (based on the parameter values listed in Table 1 at 4, 15, and 25 °C). The simulated concentration histories for the time-dependent inactivation match the experimental data slightly better than using constant inactivation rates. Sim and Chrysikopoulos (24) made a similar observation. The temporal variability of the inactivation rate coefficient has been attributed to multiphasic sequential inactivation caused by the presence of two or more subpopulations of bacteriophage that exhibit different heat sensitivities (11, 17, 25). The magnitude of  $\alpha$  is proportional to the resistivity of the dominant subpopulation because the overall inactivation is



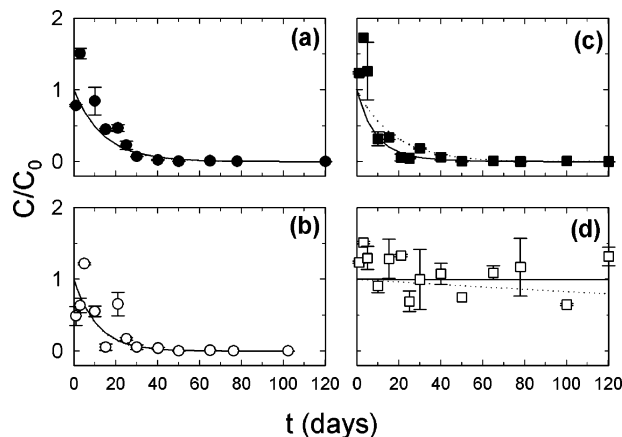
**FIGURE 1. Experimental data for MS2 inactivation under static batch conditions with sand (solid circles) and without sand (open circles) at temperatures of (a, d) 4 °C, (b, e) 15 °C, and (c, f) 25 °C. Simulated concentration histories are based on the time-dependent inactivation model (solid curves) and constant rate inactivation model (dotted curves).**



**FIGURE 2. Experimental data for PRD1 inactivation under static batch conditions with sand (solid squares) and without sand (open squares) at temperatures of (a, d) 4 °C, (b, e) 15 °C, and (c, f) 25 °C. Simulated concentration histories are based on the time-dependent inactivation model (solid curves) and constant rate inactivation model (dotted curves).**

controlled by the dominant subpopulation (24). Temporally variable inactivation allows for rapid inactivation of the most sensitive subpopulations and slower inactivation of the more resistive subpopulations. Note that, in the presence of sand,  $\lambda_0$  increases faster with increasing temperature than for the case where there is no sand present, whereas  $\alpha$  is greatest at 25 °C in the absence of sand.

The static batch PRD1 inactivation experimental data with and without sand, as well as the corresponding simulated concentration histories for both time-dependent and constant inactivation rates, based on the parameter values listed in Table 1 at 4, 15, and 25 °C, are shown in Figure 2. Clearly, the temperature as well as the presence of sand have only a minor effect on PRD1 inactivation under the conditions examined in this work. Note that, for PRD1, the observed

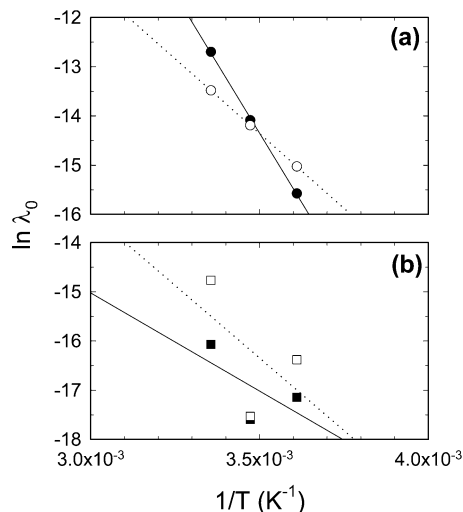


**FIGURE 3.** Experimental data for (a, b) MS2 and (c, d) PRD1 inactivation under dynamic batch conditions with sand (solid symbols) and without sand (open symbols) at 15 °C. Simulated concentration histories are based on the time-dependent inactivation model (solid curves) and constant rate inactivation model (dotted curves).

greater inactivation at 4 °C than at 15 °C suggests a minor effect of temperature might exist during the inactivation process. It should be noted that  $\lambda_0$  for PRD1 in the presence of sand is affected by temperature to a lesser extent than the case where there is no sand present. The increased  $\alpha$  value for PRD1 at 25 °C in the absence of sand indicates the presence of a large subpopulation that is relatively insensitive to heat.

Figure 3 shows the bacteriophage inactivation experimental data from the dynamic batch experiment at 15 °C with and without sand, together with the corresponding simulated concentration histories for both time-dependent and constant inactivation rates. On the basis of the similar  $\lambda_0$  and  $\lambda$  values for the static and dynamic batch experiments at 15 °C listed in Table 1, the presence of an air phase has little effect on the inactivation process of the dominant MS2 subpopulation. Thompson et al. (32) observed a similar lack of MS2 inactivation under similar batch conditions. However, there is a slight increase in  $\alpha$  values during the dynamic batch experiments, especially in the presence of sand. Such an increase in the magnitude of  $\alpha$  suggests that a subpopulation of MS2 bacteriophage exists with a slightly higher sensitivity to the presence of an air phase. In contrast, the increased inactivation rate for PRD1 during the dynamic batch experiment with sand, along with the increased  $\alpha$  values with and without sand, suggest that the presence of air-liquid and air-solid interfaces, instead of temperature, sand, or the presence of air-liquid interfaces, are responsible for the greatest damage to specific viral components that are required for infection.

**Inactivation Thermodynamics.** The native structure of proteins is maintained by a complex interplay of various forces that act to fold and hold their primary structure, the polypeptide chain, in a specific conformation (11). Therefore, the molecular process of virus inactivation occurs when protein conformation is altered or destroyed by some environmental stress and the altered protein molecules lose their ability to infect the host cell. To analyze the molecular processes involved in the inactivation process, the  $\lambda_0$  values listed in Table 1 were used to determine the thermodynamic parameters  $E_a$ ,  $\Delta G^\ddagger$ ,  $\Delta H^\ddagger$ , and  $\Delta S^\ddagger$  for each of the bacteriophages considered in this study. It should be noted that these parameters describe the thermodynamics of the formation of the activated (transition) state shown in eq 7 and, as such, provide information on the energy requirements to undergo the inactivation process. Equilibrium values for virus inactivation are required to obtain thermodynamic parameters for the complete inactivation process shown in eq 7. However,



**FIGURE 4.** Arrhenius plots for (a) MS2 and (b) PRD1 inactivation under static batch conditions with sand (solid symbols) and without sand (open symbols). The slopes of the Arrhenius plots indicate the activation energies in the presence of sand (solid line) and in the absence of sand (dotted line).

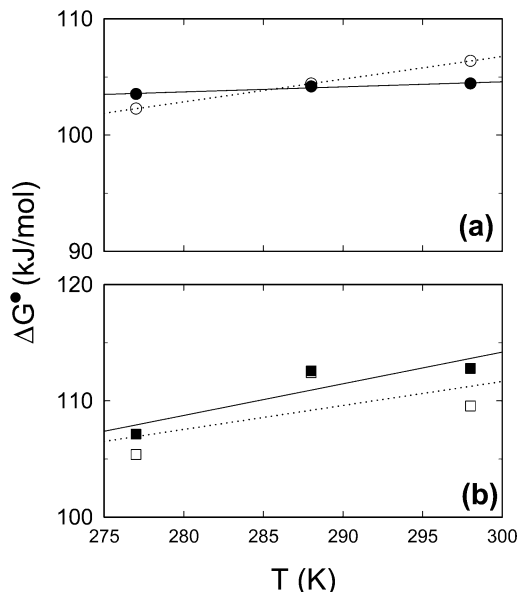
**TABLE 2.** Thermodynamic Parameters for Bacteriophage MS2 and PRD1 Inactivation

	$E_a$ (kJ/mol)	$\Delta G^\ddagger$ (kJ/mol) 4, 15, and 25 °C	$\Delta H^\ddagger$ (kJ/mol)	$\Delta S^\ddagger$ (J/mol·K)
<b>MS2</b>				
sand	93.9	103.5, 104.2, 104.4	91.6	43.3
no sand	50.5	102.3, 104.4, 106.4	48.1	195.5
<b>PRD1</b>				
sand	33.2	107.2, 112.6, 112.8	32.4	272.5
no sand	49.0	105.4, 112.42, 109.6	49.8	206.2

it is not possible to obtain these values because the alteration that causes virus inactivation is irreversible or, at least, difficult to reverse (11).

Arrhenius plots for MS2 and PRD1 inactivation for the static batch experiments with and without sand using  $\lambda_0$  values listed in Table 1 are shown in Figure 4. The slopes of the MS2 Arrhenius plots shown in Figure 4a indicate that the energies of activation (formation of the transition state that results in loss of infectivity) state required for MS2 to undergo the inactivation process under the static batch conditions were 93.9 kJ/mol in the presence of sand and 50.5 kJ/mol in the absence of sand. Comparison of the slopes of the MS2 Arrhenius plots with and without sand shown in Figure 4a indicates a crossover pattern with a greater  $\lambda_0$  in the absence of sand at 4 and 15 °C and a greater  $\lambda_0$  in the presence of sand at 25 °C. The slopes of the PRD1 Arrhenius plots shown in Figure 4b indicate that the energies of activation required for PRD1 to undergo the inactivation process under the static batch conditions are 33.2 kJ/mol in the presence of sand and 49.0 kJ/mol in the absence of sand. For PRD1, the  $\lambda_0$  values are greater in the absence of sand for all temperatures considered in this study. The activation energies for the inactivation of both MS2 and PRD1 are listed in Table 2. Preston and Farrah (33) reported that low activation energies ( $E_a < 160$  kJ/mol; 1 calorie = 4.184 J) indicate that virus adsorption is a physical process rather than a chemical process. The low activation energies for both bacteriophages employed in this study suggest that electrostatic and/or hydrodynamic interactions play a significant role in virus inactivation. However, the standard entropy of formation of the activated state is also an important factor in determining the magnitude of the inactivation rate coefficient (11).





**FIGURE 5.** Free energy of formation of the activated (transition) state,  $\Delta G^\circ$ , as a function of temperature for (a) MS2 and (b) PRD1 inactivation under static batch conditions with sand (solid symbols) and without sand (open symbols). The slopes of the free energy plots indicate the standard entropy of formation of the activated (transition) state,  $\Delta S^\circ$ , and the  $y$ -intercepts indicate the standard enthalpy of formation of the activated (transition) state,  $\Delta H^\circ$ , in the presence of sand (solid line) and in the absence of sand (dotted line).

In view of the relationship in eq 12, it is clear that the standard entropy of formation of the activated (transition) state,  $\Delta S^\circ$ , can be determined directly from the change in  $\Delta G^\circ$  values as a function of temperature, where the  $y$ -intercepts indicate the standard enthalpy of formation of the activation (transition) state,  $\Delta H^\circ$ . The  $\Delta G^\circ$  values for the inactivation of both MS2 and PRD1 under static batch conditions at 4, 15, and 25 °C were determined from eq 13, and they are listed in Table 2. Figure 5 presents the free energy plots for MS2 and PRD1 inactivation with and without sand. For MS2, it was determined from the slope of the free energy plot shown in Figure 5a that  $\Delta S^\circ$  is equal to 43.3 J/mol·K in the presence of sand and 195.5 J/mol·K in the absence of sand. For PRD1, it was determined from Figure 5b that  $\Delta S^\circ$  is equal to 272.5 J/mol·K in the presence of sand and 206.1 J/mol·K in the absence of sand. The  $\Delta S^\circ$  values listed in Table 2 indicate the presence of sand has a greater effect on increasing disorder of the activated (transition) state for PRD1 (value is more positive) than it is for MS2. This results in lower  $\Delta G^\circ$  values for MS2 and allows its inactivation process to proceed at a faster rate than that of PRD1. In contrast, the absence of sand has the effect of decreasing slightly the  $\Delta S^\circ$  value for PRD1. It should be noted here that the results of the static batch experiments performed during this study provide similar  $\Delta S^\circ$  and  $\Delta H^\circ$  values to those reported by Ginoza (11) at higher temperatures and suggest that protein denaturation is the predominant mechanism involved in the inactivation process of viruses.

The results from the dynamic batch experiments suggest that the presence of air–liquid and air–solid interfaces has the effect of decreasing  $\Delta S^\circ$  values for PRD1 during the inactivation process as well. However, it is not clear from the kinetic data collected during the dynamic batch experiments whether protein denaturation is the cause of inactivation. Using thermodynamic parameters based on the time-dependent inactivation model provides a better method to predict the inactivation of viruses in groundwater. Furthermore, the observed temporal variation of the inactivation

rate coefficients suggest that virus subpopulations may remain infective in porous media for an extended period of time and continue to migrate downstream to points of withdrawal.

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## Literature Cited

- (1) Yates, M. V.; Gerba, C. P.; Kelley, L. M. Virus persistence in groundwater. *Appl. Environ. Microbiol.* **1985**, *49*, 778–781.
- (2) Chrysikopoulos, C. V.; Sim, Y. One-dimensional virus transport in homogeneous porous media with time-dependent distribution coefficient. *J. Hydrol.* **1996**, *185*, 199–219.
- (3) Gerba, C. P.; Keswick, B. H. Survival and transport of enteric bacteria and viruses in groundwater. *Stud. Environ. Sci.* **1981**, *17*, 511–515.
- (4) Sim, Y.; Chrysikopoulos, C. V. Analytical models for virus adsorption and inactivation in unsaturated porous media. *Colloids Surf., A* **1999**, *155*, 189–197.
- (5) Gerba, C. P. Applied and theoretical aspects of virus adsorption to surfaces. *Adv. Appl. Microbiol.* **1984**, *30*, 133–168.
- (6) Hurst, C. J.; Gerba, C. P.; Cech, I. Effects of environmental variables and soil characteristics on virus survival in soil. *Appl. Environ. Microbiol.* **1980**, *40*, 1067–1079.
- (7) Keswick, B. H.; Gerba, C. P. Viruses in groundwater. *Environ. Sci. Technol.* **1980**, *14*, 1290–1297.
- (8) Sobsey, M. D.; Dean, C. H.; Knuckles, M. E.; Wagner, R. A. Interactions and survival of enteric viruses in soil materials. *Appl. Environ. Microbiol.* **1980**, *40*, 92–101.
- (9) Jansons, J.; Edmonds, L. W.; Speight, B.; Bucens, M. R. Survival of viruses in groundwater. *Water Res.* **1989**, *23*, 301–306.
- (10) Yates, M. V.; Yates, S. R.; Wagner, J.; Gerba, C. P. Modeling virus survival and transport in the subsurface. *J. Contam. Hydrol.* **1987**, *1*, 329–345.
- (11) Ginoza, W. In *Methods in Virology*; Maramorosch, K., Koprowski, H., Eds.; Academic Press: New York, 1968; pp 139–209.
- (12) Yates, M. V.; Ouyang, Y. VIRTUS, a model of virus transport in unsaturated soils. *Appl. Environ. Microbiol.* **1992**, *58*, 1609–1616.
- (13) Sim, Y.; Chrysikopoulos, C. V. Analytical models for one-dimensional virus transport in saturated porous media. *Water Resour. Res.* **1995**, *31*, 1429–1437. Correction, *Water Resour. Res.* **1996**, *32*, 1473.
- (14) Parkinson, J. S.; Huskey, R. J. Deletion mutants of bacteriophage lambda. I. Isolation and initial characterization. *J. Mol. Biol.* **1971**, *56*, 369–384.
- (15) Pollard, E. C.; Solosko, W. The thermal inactivation of T<sub>4</sub> and λ bacteriophage. *Biophys. J.* **1971**, *11*, 66–74.
- (16) Yamagishi, H.; Ozeki, H. Comparative study of thermal inactivation of phage φ80 and lambda. *Virology* **1972**, *48*, 316–322.
- (17) Grant, S. B.; List, E. J.; Lidstrom, M. E. Kinetic analysis of virus adsorption and inactivation in batch experiments. *Water Resour. Res.* **1993**, *29*, 2067–2085.
- (18) Yahya, M. T.; Galsomies, L.; Gerba, C. P.; Bales, R. C. Survival of bacteriophage MS-2 and PRD-1 in ground water. *Water Sci. Technol.* **1993**, *27*, 409–412.
- (19) Ryan, J. N.; Harvey, R. W.; Metge, D.; Elimelech, M.; Navigato, T.; Pieper, A. P. Field and laboratory investigations of inactivation of viruses (PRD1 and MS2) attached to iron oxide-coated quartz sand. *Environ. Sci. Technol.* **2002**, *36*, 2403–2413.
- (20) Harvey, R. W.; Ryan, J. N. Use of PRD1 bacteriophage in groundwater viral transport, inactivation, and attachment studies. *FEMS Microbiol. Ecol.* **2004**, *49*, 3–16.
- (21) Anders, R.; Chrysikopoulos, C. V. Virus fate and transport during artificial recharge with recycled water. *Water Resour. Res.* **2005**, *41*, W10415, <http://dx.doi.org/10.1029/2004WR003419>.
- (22) Adams, M. H. *Bacteriophages*; Interscience Publishers: New York, 1959.

- (23) DeBartolomeis, J.; Cabelli, V. J. Evaluation of an *Escherichia coli* host strain for enumeration of male-specific bacteriophages. *Appl. Environ. Microbiol.* **1991**, *57*, 1301–1305.
- (24) Sim, Y.; Chrysikopoulos, C. V. One-dimensional virus transport in porous media with time-dependent inactivation rate coefficients. *Water Resour. Res.* **1996**, *32*, 2607–2611.
- (25) Chrysikopoulos, C. V.; Vogler, E. T. Estimation of time dependent virus inactivation rates by geostatistical and resampling techniques: Application to virus transport in porous media. *Stochastic Environ. Res. Risk Assess.* **2004**, *18*, 67–78.
- (26) Morel, F. M. M.; Hering, J. G. *Principles and Applications of Aquatic Chemistry*; John Wiley and Sons: New York, 1993.
- (27) Atkins, P. W. *Physical Chemistry*, 4th ed.; Oxford University Press: New York, 1990.
- (28) Murray, J. P.; Laband, S. J. Degradation of poliovirus by adsorption on inorganic surfaces. *Appl. Environ. Microbiol.* **1979**, *37*, 480–486.
- (29) Grant, S. B. Inactivation Kinetics of Viral Aggregates. *J. Environ. Eng.* **1995**, *121*, 311–319.
- (30) Chu, Y.; Jin, Y.; Flury, M.; Yates, M. V. Virus transport through saturated sand columns as affected by different buffer solutions. *J. Environ. Qual.* **2000**, *29*, 1103–1110.
- (31) Matsui, Y.; Matsushita, T.; Sakuma, S.; Gojo, T.; Mamiya, T.; Suzuoki, H.; Inoue, T. Virus inactivation in aluminum and polyaluminum coagulation. *Environ. Sci. Technol.* **2003**, *37*, 5175–5180.
- (32) Thompson, S. S.; Yates, M. V. Bacteriophage inactivation at the air-water-solid interface in dynamic batch systems. *Appl. Environ. Microbiol.* **1999**, *65*, 1186–1190.
- (33) Preston, D. R.; Farrah, S. R. Activation thermodynamics of virus adsorption to solids. *Appl. Environ. Microbiol.* **1988**, *54*, 2650–2654.

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