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Inactivation of Enteric Adenovirus and Feline Calicivirus by Chlorine Dioxide

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Chlorine dioxide (ClO2) inactivation experiments were conducted with adenovirus type 40 (AD40) and feline calicivirus (FCV). Experiments were carried out in buffered, disinfectant demand-free water under high- and low-pH and -temperature conditions. Ct values (the concentration of ClO2 multiplied by contact time with the virus) were calculated directly from bench-scale experiments and from application of the efficiency factor Hom (EFH) model. AD40 Ct ranges for 4-log inactivation (Ct99.99%) at 5°C were >0.77 to <1.53 mg/liter × min and >0.80 to <1.15 mg/liter × min for pH 6 and 8, respectively. For 15°C AD40 experiments, >0.49 to <0.74 mg/liter × min and <0.12 mg/liter × min Cl99.99% ranges were observed for pH 6 and 8, respectively. FCV Ct99.99% ranges for 5°C experiments were >20.20 to <30.30 mg/liter × min and >0.68 mg/liter × min for pH 6 and 8, respectively. For 15°C FCV experiments, Ct99.99% ranges were >4.20 to <6.72 and <0.18 mg/liter × min for pH 6 and 8, respectively. Viral inactivation was higher at pH 8 than at pH 6 and at 15°C than at 5°C. Comparison of Ct values and inactivation curves demonstrated that the EFH model described bench-scale experiment data very well. Observed bench-scale Cl99.99% ranges and EFH model Ct99.99% values demonstrated that FCV is more resistant to ClO2 than AD40 for the conditions studied. U.S. Environmental Protection Agency guidance manual Cl99.99% values are higher than Cl99.99% values calculated from bench-scale experiments and from EFH model application.

According to the U.S. Environmental Protection Agency (EPA) National Primary Drinking Water Standards, enteric viruses must be removed or inactivated by 4 logs (99.99%) from source water by filtration and disinfection or by a combination of these technologies (48). Viral pathogens can bypass conventional filtration processes due to their small size, making disinfection an important treatment barrier between drinking water consumers and viral gastroenteritis. While chlorine is the most common disinfectant in the United States for drinking water and wastewater treatment, alternative disinfectants are needed to reduce highly chlorine resistant pathogens, such as Cryptosporidium parvum. UV light disinfection is effective at reducing Cryptosporidium oocysts (6, 34), and it is therefore an attractive alternative disinfectant to chlorine for drinking water treatment. Recent evidence, however, has shown that UV light is ineffective at reducing enteric adenovirus at doses commonly applied by water treatment systems (45). Furthermore, UV light water disinfection leaves no residual for protection of potable water after it leaves the treatment plant. To effectively reduce viruses by 99.99% and to maintain a disinfectant residual in the distribution system, a secondary disinfectant is needed.

ClO2 is an alternative to chlorine as a primary disinfectant or can serve as a secondary disinfectant for UV treatment systems. Advantages of ClO2 disinfection include (i) oxidation of iron and manganese (reduces discoloration of finished water), (ii) no trihalomethane formation, (iii) no reaction with ammonia, (iv) less affected by the pH conditions typical of drinking water than is chlorine, (v) a relatively persistent residual, and (vi) reduction of tastes and odors caused by organic and sulfuriferous compounds (1, 5). Disadvantages of ClO2 disinfection include (i) formation of organic halides, (ii) formation of chlorite and chlorate, and (iii) production of taste and odors at concentrations of >0.5 mg/liter (5). However, this strong oxidant is a useful and attractive alternative to chlorine for the advances listed above and its reported increased ability to reduce pathogenic microorganisms in water. Previous studies have reported that ClO2 effectively inactivates several viruses in water and sewage (14, 21, 39, 43), but limited information is available concerning the reduction of caliciviruses and adenoviruses in drinking water.

The EPA, mandated by the Safe Drinking Water Act, published the Drinking Water Contaminant Candidate List (CCL) in 1998 (12). This list includes chemical and microbial contaminants that are known or anticipated to occur in public water systems. These contaminants are under regulatory consideration, since little to no information regarding health, drinking, wastewater treatment, or analytical methodology is currently available. Enteric viruses and caliciviruses are included in the CCL and were investigated in this study.

Members of the human calicivirus genus, noroviruses (NVs), are a principal cause of nonbacterial acute gastroenteritis (11, 28) and have been identified as etiological agents of waterborne outbreaks (20, 29, 31, 32). Caliciviruses range in diam-
eter from 27 to 40 nm and have a single-stranded RNA genome and an icosahedral capsid structure. Commonly reported symptoms include diarrhea and vomiting. Previous outbreaks caused by NV-contaminated ice and cooked shellfish have suggested that these viruses are capable of withstanding harsh environmental conditions (16). Their ability to withstand current drinking water disinfection practices is largely unknown, since there are no known animal or mammalian cell culture systems that determine NV infectivity. Due to these difficulties, two alternative studies, a human feeding study and a PCR-based study, were carried out previously (30, 41). However, conflicting results between these studies made conclusions regarding NV chlorine resistance difficult. More recently, an NV surrogate, feline calicivirus (FCV), has been used as a surrogate for NV inactivation in several disinfection studies (9, 36, 42, 44, 45). Since FCV has genome organization (7, 26) and capsid architecture (38) similar to those of NVs and can be easily grown in cell culture, it is an appropriate surrogate for NV. Chlorine inactivation experiments carried out with FCV resulted in conclusions similar to those reported in the NV PCR-based study. Results from these two studies suggest that chlorine is effective at reducing these caliciviruses by 4 logs at commonly applied chlorine concentrations (44).

Like NVs, the enteric adenoviruses, enteric adenovirus 40 (AD40) and AD41, are also important causes of self-limiting, acute gastroenteritis, especially in children <4 years of age (23). Ranging from 70 to 90 nm in size, these viruses are considerably larger than noroviruses, and their capsid structure is complex. The adenovirus icosahedron contains 240 hexons, 12 pentons, and 12 fibers that extend from each penton base; its genome consists of linear, double-stranded DNA. Enteric adenoviruses are shed in high numbers in the feces (2), are typically shed in the feces for long periods, and infection can be caused by low numbers of viral particles (16, 23). Enteric adenoviruses have greater environmental stability than other enteric viruses (10), so their presence in sewage and surface water makes them likely contaminants in public water supplies (24, 25). Moreover, enteric adenoviruses and noroviruses were identified as two of the etiological agents causing acute gastroenteritis in a waterborne outbreak in Finland (31), and waterborne outbreaks of pharyngoconjunctivitis from swimming have been reported for nonenteric adenoviruses (13, 37). Enteric adenoviruses are susceptible to chlorine (44) but are very resistant to UV light (45).

Based on previous viral disinfection studies, the EPA published the Guidance Manual for Compliance with the Filtration and Disinfection Requirements for Public Water Sources (46). Ct values, which are the disinfectant concentration (C) multiplied by the contact time (t) between the disinfectant and microorganism, for 2- to 4-log viral inactivation by ClO2 and other water disinfectants at different pH and temperature conditions are listed in the manual. Ct values for viral inactivation are based on experiments conducted with hepatitis A virus (HAV). The guidance manual’s Ct values (in milligrams per liter, multiplied by the number of minutes) direct public water utilities to ensure that disinfection practices meet regulatory microbial log inactivation requirements. However, ClO2 Ct values may not be adequate for caliciviruses and adenoviruses whose susceptibility to this disinfectant is largely unknown.

The objectives of this study were to (i) compare viral inactivation by ClO2 for AD40 and FCV in water under high- and low-pH (pH 8 and 6) and -temperature (15°C and 5°C) conditions, (ii) use a previously described disinfection model to determine Ct values for each virus and experimental condition, and (iii) compare predicted Ct values to the EPA guidance manual Ct values and disinfection practices commonly applied in the United States.

### MATERIALS AND METHODS

**Virus propagation and assay.** AD40 (strain Dugan), FCV (strain F9), primary liver carcinoma cell line (PLC/PRF/S), and Crandell Reese feline kidney cell lines were obtained from the American Type Culture Collection (Rockville, MD). AD40 and FCV stocks were propagated, enumerated, concentrated, and purified to reduce disinfection demand in the same manner described by Thurston-Enriquez et al. (45). All viral stocks were stored at 4°C until use. Determination of viral titer before and after chlorine disinfection was accomplished by assaying 5- to 10-fold dilutions in quadruplicate in 24-well tissue culture trays with the appropriate cells in suspension (44).

**ClO2 production and measurement.** ClO2 was generated using the iodometric method (4). ClO2 concentrations of the stock solution and in buffered, demand-free (BDF) water throughout disinfection experiments were measured according to Hach (Loveland, CO) DPD method 10126 with a Hach DR2000 spectrophotometer.

**Experimental protocol.** Glassware and BDF water were prepared. The experimental protocol was carried out according to protocols used by Thurston-Enriquez et al. (45). Briefly, BDF water was kept at a constant temperature (5°C or 15°C) in a refrigerated water bath. ClO2 was added at a volume necessary to achieve an initial disinfectant dose close to 0.50 mg/liter or 1.0 mg/liter. Chlorine dioxide doses applied in this study’s disinfection experiments ranged from 0.20 to 200 mg/liter.

<table>
<thead>
<tr>
<th>Virus</th>
<th>BDF water conditions</th>
<th>No. of replicates</th>
<th>k (min⁻¹)</th>
<th>n</th>
<th>m</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD40 0.51</td>
<td>5 6</td>
<td>2</td>
<td>0.03</td>
<td>8.32</td>
<td>0.62</td>
</tr>
<tr>
<td>AD40 0.53</td>
<td>5 8</td>
<td>2</td>
<td>0.04</td>
<td>362.0</td>
<td>6.01</td>
</tr>
<tr>
<td>AD40 0.49</td>
<td>15 6</td>
<td>2</td>
<td>0.10</td>
<td>5.61</td>
<td>0.01</td>
</tr>
<tr>
<td>FCV 1.01</td>
<td>5 6</td>
<td>5</td>
<td>0.03</td>
<td>1.59</td>
<td>0.01</td>
</tr>
<tr>
<td>FCV 0.90</td>
<td>5 8</td>
<td>4</td>
<td>0.03</td>
<td>8.58</td>
<td>0.01</td>
</tr>
<tr>
<td>FCV 0.84</td>
<td>15 6</td>
<td>4</td>
<td>0.05</td>
<td>2.20</td>
<td>0.01</td>
</tr>
<tr>
<td>FCV 0.72</td>
<td>15 8</td>
<td>3</td>
<td>0.07</td>
<td>167.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

EFH model parameters k, n, and m are dimensionless.

aAverage ClO2 concentration applied in replicate experiments.

bViruses not detected by cell culture assays after 15 s of contact time and ≥4.21-log inactivation.

cViruses not detected by cell culture assays after 15 s of contact time and ≥4.15-log inactivation.
representative of viral concentrations per milliliter of BDF water in beakers two
and three. This control beaker was necessary to (i) determine the initial virus
concentration for every experiment and (ii) evaluate whether virus inactivation
occurred under the tested BDF water, pH, and temperature conditions (in the
absence of disinfectants). Viral samples were kept on ice during the experiment
and then stored at 4°C until assay.

Kinetic modeling and Ct values. Chlorine decay constants (k’) for each experi-
ment were calculated using the Solver function in Microsoft Excel 2000 (Mi-
crosoft Corp.) to regress the first-order kinetic equation (equation 1) using the
least-squares method.

\[
C = C_0 \exp(-k't)
\]  

where \( C \) and \( C_0 \) are the ClO2 residual (in milligrams per liter) at time \( t \) (in
minutes) and time 0.25 min (the closest possible measurement to time zero),
respectively; \( k' \) is the first-order disinfectant decay rate constant (per minute)
(19). Disinfectant decay (k’) values are listed in Table 1 for each set of disin-
fection experiments.

Efficiency factor Hom (EFH) model parameters (Table 1) and Ct values
(Table 2) were calculated by applying the EFH model (equation 2) to data
obtained from bench-scale disinfection experiments (19). The EFH model is an
analytical approximation of the incomplete gamma Hom (IGH) model. These
models are considered to adequately describe the kinetics of disinfection ex-
periments that do not follow Chick-Watson relationships and are subject to disin-
fectant decay (19). Unlike the IGH model, the EFH model enables researchers
to describe disinfection kinetics by using mathematical functions available in
commonly used computer packages such as Microsoft Excel (Microsoft Corp.)
(19). IGH and EFH models have been employed to describe disinfection kinetics
in previous studies (17, 18, 33) and have been used to predict enteric adenovirus
type 40 and feline calicivirus inactivation by chlorine (44).

Viral most-probable-number values for each experiment, grouped by virus
type, pH, and temperature conditions, were fit into the EFH model (equation 2)

\[
\ln N/N_0 = -kCt^m \times [1 - \exp(-nk't/m)]/(nk't/m)
\]  

where \( r \) is exposure time (min), \( k \) is the viral inactivation rate constant (dimen-
sionless), \( n \) is the coefficient of dilution (dimensionless), \( k' \) is the first-order
inactivating disinfectant decay rate constant (per minute), and \( m \) is the constant for the
inactivation rate law which describes deviation from ideal Chick-Watson kinetics
(dimensionless) (13). In \( N/N_0 \) is the natural log of the survival ratio (the number of
viruses remaining at time \( r \) divided by the initial viral concentration). Microsoft
Solver (Microsoft Excel 2000; Microsoft Corp.) was used to minimize the sum
of squares of the difference between the observed and \( N/N_0 \) value for viral
disinfection experiments performed with the same virus and conditions, to de-
termine the EFH model coefficients for each viral and set of conditions (Table 1). Using
GraphPad Prism version 4.00 for Windows (San Diego, CA), average viral
concentrations versus time were charted for observed viral inactivation and for
values fitted by the EFH model (Fig. 1 and 2).

A Ct value is determined by multiplying the disinfectant concentration (C) in
milligrams per liter by the time (t) in minutes when a specific log inactivation (2,
3, or 4 log; 99%, 99.9%, or 99.99%) occurred. Ct values (Ct99.0%, Ct99.9%, and
Ct99.99%) were used to assess viral sensitivity to ClO2 and evaluate the ability of
the EFH model to fit data obtained from bench-scale viral inactivation experi-
ments (Table 2). EFH model Ct99.99% values were determined through applica-
tion of EFH model parameters (Table 1). A value of 0.0001 for \( k' \) (conditions of
negligible disinfectant decay) was used for EFH Ct values. This value was chosen
to produce baseline Ct values and because \( k' \) varied between experiments. The
average ClO2 dose applied in replicate experiments was also used to determine
EFH model Ct values. Ct value ranges were calculated by multiplying the average
ClO2 concentration applied for each set of replicate experiments by the closest
time points to 4-log viral inactivation. For example, AD40 experiments con-
ducted at pH 8 and 5°C had an average log inactivation of 3.41 and 4.21 at 1.5
min and 3 min, respectively. The average ClO2 dose for this set of experiments
was 0.53 mg/liter. The range, therefore, is >0.80 to <1.59 mg/liter × min.

**Statistical analysis.** Average microbial concentrations and standard deviations
were calculated and graphed using GraphPad Prism, version 4.00, for Windows
(San Diego, CA). F tests were carried out using Microsoft Excel 2000 (Microsoft
Corporation).

**TABLE 2. AD40 and FCV Ct99.99% ranges observed from bench-scale inactivation experiments, Ct99.99% values calculated by fitting the EFH model to bench-scale data, and EPA guidance manual Ct99.99% values**

<table>
<thead>
<tr>
<th>BDF water conditions</th>
<th>AD40 observed ranges</th>
<th>AD40 EFH modelb</th>
<th>FCV observed ranges</th>
<th>FCV EFH modelb</th>
<th>EPA guidance manual</th>
</tr>
</thead>
<tbody>
<tr>
<td>°C</td>
<td>pH</td>
<td>Ct99.99% (mg/liter × min)</td>
<td>Ct99.99% (mg/liter × min)</td>
<td>Ct99.99% (mg/liter × min)</td>
<td>Ct99.99% (mg/liter × min)</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>&gt;0.77 to &lt;1.53</td>
<td>1.28</td>
<td>&gt;20.20 to &lt;30.30</td>
<td>20.85</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>&gt;0.80 to &lt;1.59</td>
<td>0.67</td>
<td>&gt;0.68 (3.60 log)c</td>
<td>1.08</td>
</tr>
<tr>
<td>15</td>
<td>6</td>
<td>&gt;0.49 to &lt;0.74</td>
<td>0.92</td>
<td>&gt;4.20 to &lt;6.72</td>
<td>7.13</td>
</tr>
<tr>
<td>15</td>
<td>8</td>
<td>&lt;0.12</td>
<td>0.11</td>
<td>&lt;0.18d</td>
<td>0.19</td>
</tr>
</tbody>
</table>

\( ^a \) Ct values calculated using \( k' = 0.00001 \) (conditions of negligible disinfectant decay).

\( ^b \) Duration of experiment was not long enough to achieve 99.99% inactivation. Ct value corresponds to 3.60-log inactivation by 45 s.

\( ^c \) Value is ≥4.21-log inactivation by 15 s.

\( ^d \) Value is ≥4.15-log inactivation by 15 s.

**FIG. 1. Observed and EFH model AD40 ClO2 inactivation curves (ClO2 doses ranged from 0.47 to 0.53 mg/liter in buffered, demand-free water).**
RESULTS

ClO₂ disinfection experiments were carried out in at least in duplicate for AD40 and FCV under high- and low-pH and -temperature conditions in BDF water. The EFH model was used to model disinfectant inactivation kinetics for every experimental condition applied to each virus. Table 1 lists BDF water conditions applied to viral disinfection experiments and parameter estimates for EFH model analysis.

Table 2 compares $C_{99.99\%}$ ranges observed from bench-scale inactivation experiments, $C_{99.99\%}$ values calculated by fitting the EFH model to bench-scale data, and EPA guidance manual $C_{99.99\%}$ values. The EFH model fit the observed bench-scale data well, producing $C_{99.99\%}$ values close to or within the range of observed $C_{99.99\%}$ values. AD40 and FCV EFH $C_{99.99\%}$ values were lower than $C_{99.99\%}$ values recommended in the EPA guidance manual for viral inactivation in water.

When observed $C_{99.99\%}$ ranges and EFH $C_{99.99\%}$ values were compared, it was noted that FCV is more resistant to ClO₂ than FCV for most of the conditions studied. Differences in viral sensitivities under pH 8 and 15°C conditions, however, are unclear, since AD40 and FCV were completely inactivated by the first sample collection taken at 15 s. For all other tested conditions, FCV appears to be much more resistant to ClO₂ than AD40.

The $C_{99.99\%}$ ranges calculated for viral inactivation are difficult to compare when 4-log inactivation was not observed. At pH 8 and 5°C, the FCV $C_{99.99\%}$ range is >0.68, since only 3.60-log inactivation was observed at the last time point sampled. For this set of experiments, 3.60 log was was inactivated within 45 s. For FCV inactivation at pH 6 and 5°C, however, an average of only 0.90 logs was inactivated by 1 min. Thus, FCV appears to be more resistant at pH 6 than at pH 8. This difference is better reflected by comparing EFH model $C_{99.99\%}$ values where the difference in inactivation rates is obvious between FCV inactivation at pH 6 (20.85 mg/liter × min) and pH 8 (1.08 mg/liter × min).

Observed bench-scale viral inactivation curves and EFH model inactivation curves for all tested water conditions are shown in Fig. 1 and 2. Data points listed as observed in the charts are average viral concentrations from replicate bench-scale experiments. Fitting bench-scale viral inactivation data into the EFH model generated curves listed as EFH. Significant differences ($P < 0.05$) in viral inactivation rates for high- and low-temperature and -pH conditions were observed. For AD40 experiments conducted at 5°C, inactivation rates are not significant, starting at the 1-min time point (Fig. 1). However, the rate of AD40 inactivation was higher under pH 8 conditions than under pH 6 conditions for the first 30 s. Tailing of the curves was similar, resulting in insignificant inactivation from the 1-min to the 3-min time point. Viral inactivation rates were higher for experiments carried out at pH 8 than at pH 6 (AD40, 15°C; FCV, 5 and 15°C) and 15°C than at 5°C.

DISCUSSION

To our knowledge, this is the first report of ClO₂ inactivation of enteric adenovirus and generation of Cl₉ values for ClO₂ inactivation of AD40 and FCV in water at high- and low-pH and -temperature conditions. This information is important not only for evaluation of ClO₂ as a secondary disinfectant for water systems employing UV light but also for systems utilizing ClO₂ as a primary disinfectant. $C_{99.99\%}$ values, calculated based on bench-scale experiments and those predicted by the EFH model for AD40 and FCV, suggest that EPA guidance manual Cl₉ values (46) are sufficient for reducing these viruses in treated water under the temperature and pH conditions tested by this study. Considering that the range in ClO₂ dosage employed by the United States water industry is 0.07 to 2.0 mg/liter (47) and that the average contact time is 237 min (derived from water treatment plants employing chlorination) (49), the studied viruses would be inactivated by at least 4 logs for the majority of water conditions studied. However, using the average contact time (237 min), the range in Cl₉ values would be from 16.59 to 474 mg/liter × min for water systems in the United States. The $C_{99.99\%}$ value for FCV in BDF water at pH 6 and 5°C, however, was within this range ($C_{99.99\%} = 20.85$ mg/liter × min).

Viral inactivation kinetics varies between different viral types, disinfectants, and water disinfection conditions. EFH model $C_{99.99\%}$ values for ClO₂ inactivation experiments at 5°C were 1.28 and 0.67 mg/liter × min for AD40 and 20.85 and 1.08...
mg/liter × min for FCV for BDF water at pH 6 and 8, respectively. Thus, Ct_{99.99\%} values for AD40 and FCV were 1.9 and 19.3 times higher at pH 6 than at pH 8, respectively. Previous studies have also shown that the potency of ClO2 is increased at higher pH levels. It has been reported that poliovirus type 1 is inactivated by ClO2 4.6 times faster at pH 9 than at pH 7 (8). In related studies, poliovirus (3), coliphage F2 (35), and Norwalk (norovirus) virus, poliovirus, and coliphage MS-2 (41) were more rapidly inactivated by ClO2 at pH 10 than at pH 6.

The rate of microbial inactivation generally increases by a factor of 2 or 3 as temperature increases by 10°C (22). Results generated by Cronier et al. (8) demonstrate that poliovirus type 1 was more rapidly inactivated in pH 7 BDF water at 15°C than at 5°C. Inactivation curves illustrated that poliovirus was roughly 3.5 times more resistant to ClO2 at 5°C than at 15°C (8). Similar to AD40 and FCV inactivation by chlorine (44), the disinfection efficiency of ClO2 increased at higher experimental temperatures. At pH 6 and 5°C, the Ct_{99.99\%} value was 1.39 and 2.9 times higher than pH 6 and 15°C for AD40 and FCV, respectively.

ClO2 Ct values for AD40 and FCV are higher than those reported for chlorine at pH 6 and 8 (44). This is a contradiction of earlier reports that demonstrated increased viral inactivation by ClO2 at high pH levels compared to chlorine (27, 39). For example, Ct_{99.99\%} values for rotavirus inactivation by chlorine and ClO2 at pH 10 and 5°C revealed that the Ct_{99.99\%} was 0.14 mg/liter × min (0.1 mg/liter chlorine dose for 1.4 min) for chlorine and ≤0.13 mg/liter × min (0.5 mg/liter ClO2 dose for 15 s or less) for ClO2 (39). Other studies, however, have reported that ClO2 and chlorine inactivation were similar for poliovirus (8) and coxsackievirus (40, 47). Similar to the results observed in the current study, Shin and colleagues (41) reported that ClO2 did not reduce poliovirus type 1, coliphage MS-2, and Norwalk virus as rapidly as free chlorine. Harakeh et al. (21) observed varying susceptibilities of viruses to different disinfectants. Coliphage F2 was more resistant to chlorine but less resistant to ClO2 than enteroviruses (coxsackievirus, echovirus, and poliovirus). Harakeh et al. (21) demonstrated that viral inactivation could differ for one virus challenged by different disinfectants or under different disinfection conditions. In the current study, FCV was more resistant to chlorine dioxide than to chlorine. For chlorine disinfection in water, however, our group observed that AD40 was more resistant than FCV. These results support early recommendations regarding the cautious use of indicator viruses as models for disinfectant efficacy (21). Proper evaluation of disinfectant efficacy should include representative enteric viruses known or thought to occur in source water and under various conditions typical of source water.

Very few studies have been conducted on enteric viral ClO2 inactivation in water. In comparison to a few of these earlier studies, AD40 inactivation appears to be comparable to other viruses. For example, AD40 Ct_{99.99\%} values fall within the Ct_{99,99\%} range for poliovirus (0.2 to 0.67 mg/liter × min) and rotavirus (0.2 to 0.3 mg/liter × min) (15, 43). In the current study, the EFH Ct_{99.99\%} value calculated for AD40 at pH 6 and 5°C was 0.38 mg/liter × min. For HAV inactivation in water (pH 6), however, it appears that HAV (Ct_{99.99\%} = 16.75 mg/liter × min) is much more resistant than AD40 (Ct_{99,99\%} = 0.83 mg/liter × min) but less resistant than FCV (Ct_{99,99\%} = 20.85 mg/liter × min) (46).

EFH Ct values provided a means for comparison of complicated data, taking into consideration replicate experiments that varied in viral concentration, disinfectant dose, disinfectant demand, and viral inactivation kinetics under different water conditions. Overall, the EFH model fit bench-scale inactivation data well for all tested conditions. EFH Ct values were within or slightly higher than Ct ranges derived from bench-scale experiments. When considering that replicate experiments varied in viral inactivation, ClO2 dose, and disinfectant decay, EFH curves modeled bench-scale inactivation data very well. The use of this model to accurately predict Ct values out of the range of bench-scale experiments, however, needs to be evaluated.

All disinfection reactions were carried out in BDF water that was inoculated with purified (removal of cell debris) and dispersed (chloroform extraction) AD40 and FCV virus stocks. The controlled disinfection reactions described in this paper provide baseline information necessary for understanding ClO2 efficacy against CCL viral pathogens in treated water under high- and low-pH and -temperature conditions. Studies of chlorine inactivation of aggregated FCV, however, reported Ct_{99.99\%} values 31.0 times higher than those observed for dispersed FCV virus particles (44). Moreover, Ct values for chlorine inactivation of AD40 and FCV in groundwater were higher than those calculated for experiments conducted with BDF water (44). Further studies are needed to determine whether EPA guidance manual ClO2 Ct values are adequate for reducing viruses in an aggregated state, associated with particulate matter, and in natural waters.

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