

The effect of chlorine demand on estimation of the inactivation rate constant

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ABSTRACT

Ct (disinfectant concentration multiplied by exposure time) values for chlorine are used by the US EPA to evaluate the efficacy of disinfection of microorganisms under various drinking water treatment conditions. These Ct values are generally derived from laboratory studies in which chlorine decay is characterized by a first order decay model. The concentration of chlorine is often only measured at the initial and final exposure times. In this study, using bacterial spore inactivation data where residual chlorine was measured at least twice in between initial and final exposure times, chlorine decay models were evaluated to determine the effect on Ct calculations. Traditionally Ct is treated as a constant in estimating the rate constant of the simple Chick-Watson inactivation kinetics model. As Ct is estimated it is subject to estimation error. To account for this error, the parameters of the chlorine decay and the inactivation models were estimated simultaneously.

Key words | chlorine, first-order decay, rate constant, second-order decay, simultaneous modelling

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INTRODUCTION

Kinetic models derived from laboratory inactivation studies conducted under oxidant demand-free conditions have routinely been used to develop Ct (C , disinfectant concentration multiplied by t , exposure time) values for various microorganisms. Frequently, chlorine decay in the bulk phase is characterized by a first-order kinetic model (Clark 1998). While buffers used in these studies can be made oxidant demand-free, it is not always possible to ensure that the microbial inoculum itself is free of material which would cause a measurable dissipation of the chlorine residual. This is particularly true when working with indigenous microbes from faecal sources or waste water effluents. Even under controlled conditions over extended exposure times chlorine residuals will degrade. This is often observed when dealing with particularly recalcitrant organisms such as protozoan cysts or bacterial spores which require longer periods of exposure to

achieve significant levels of inactivation. Various preparation procedures such as repeated washing of the microbes in demand-free buffer are useful in removing extraneous oxidant demand. However, these cleaning/-washing procedures vary in their effectiveness and, under some circumstances, such as studies designed to examine the role of microbial aggregation or particulate association, these procedures can alter the microbial inoculum under study.

With the recognition that, under many experimental conditions it is likely that chlorine residual will degrade over time, it becomes important to adequately characterize this change during the course of the experimental procedure. Quite often chlorine measurements are only determined at the beginning and end of an experiment, thus information on the changes in chlorine residual are not available. Information on the change in chlorine residual

during the course of the experiment can be very useful when the data are being used to develop Ct values under various conditions.

The concept of integrating chlorine decay models with inactivation models has been previously addressed by several investigators (Haas & Karra 1984a, b; Gyurek & Finch 1998). Gyurek & Finch (1998) noted that accounting for disinfectant disappearance during the exposure time is essential for developing optimal disinfection design criteria provided that the underlying inactivation rate law was sufficiently robust. The current study utilizes a second order modelling approach similar to that previously proposed by Clark (1998) to describe total trihalomethane formation as a function of chlorine demand.

In this report we illustrate the need to rigorously monitor chlorine levels in laboratory studies designed to determine unbiased rate constants used in the Chick-Watson inactivation model. Data are presented where chlorine residuals are taken at various time points during the course of the experiments as opposed to only being determined at the beginning and end of an experiment. These measurements allowed for a more thorough characterization of chlorine decay. Data from the inactivation experiments were used to determine Ct values derived from the estimated rate constants which simultaneously take into account microbial inactivation and disinfectant decay.

METHODS

A commercially prepared bacterial spore preparation of *Bacillus cereus* (Raven Biological Laboratories, Omaha,

Nebraska) was used in the experiments. The spore preparation had not been highly purified and was known to contain chlorine demand substances. The inactivation experiments were conducted in well-mixed batch reactors as previously described (Sivaganesan *et al.* 2006). The experimental conditions consisted of two pH levels (pH 7 and 8) over a range of four temperatures (5, 10, 15 and 23°C).

RESULTS AND DISCUSSION

Chlorine demand is usually characterized by a first-order decay model or a second order decay model (Clark 1998). The differential forms of these kinetic models are respectively given by:

$$\frac{dC}{dt} = -k_1 C \quad (1)$$

and

$$\frac{dC}{dt} = -k_2 C(C - C^*) \quad (2)$$

where k_1 , k_2 and C^* are constants. When chlorine concentrations are not measured between initial exposure time (0) and final exposure time (T), the first-order decay constant k_1 is calculated as:

$$k_1 = [\ln(C)_T - \ln(C)_0]/T \quad (3)$$

where, $\ln(C)_T$ and $\ln(C)_0$ are respectively the final and initial chlorine concentrations (in log base 10). Then for a given exposure time t ($\leq T$), the corresponding Ct value is

Table 1 | Summary of experimental conditions for the inactivation of commercially prepared *Bacillus cereus* spores with chlorine

pH	Temperature (°C)	$-\log(N_t/N_0)$	Cl_0 (mg l ⁻¹)	Time (min)	# of data sets	# of data points/data set
7	5	0–2.63	2.34	0–120	3	5,6,6
7	10	0–4.96	2.51–2.64	0–180	4	5,5,6,8
7	15	0–4.40	2.57–2.62	0–180	3	7,6,7
7	23	0–4.94	2.27–2.30	0–60	3	5,5,4
8	5	0–4.12	2.49–2.60	0–600	4	6,6,5,5
8	10	0–4.69	2.61–2.64	0–360	3	6,6,6
8	15	0–4.07	2.53–2.72	0–420	3	8,9,9
8	23	0–4.88	2.30–2.34	0–120	4	6,6,6,5

calculated as the area under the first-order decay curve and is given by:

$$Ct = \int_0^t (C)_0 e^{-k_1 \cdot x} dx = (C)_0 \cdot [1 - e^{-k_1 \cdot t}] / k_1 \quad (4)$$

where, k_1 is the calculated first-order decay constant from Equation (3). Measuring only the initial and final chlorine concentrations is not sufficient to determine the corresponding second-order kinetic model constants or Ct . Finally, if no deviation from the first order kinetics is seen in the plot of $\ln(N_0/N_t)$ vs. time, then the commonly used simple Chick-Watson model (Hoff 1986) given by:

$$\ln(N_0/N_t) = -K Ct \quad (5)$$

is used to estimate the inactivation rate constant K (in $(\text{mg} \cdot \text{min}/\text{l})^{-1}$). In Equation (5), N_0 and N_t are, respectively, the number of organisms alive at time zero and t . As there is no estimation error in the calculated Ct (Equation (4)), it is treated as a constant in the above model.

The main goal of this study was to fit an appropriate decay model for the residual chlorine–time profile. As a linear trend was seen in the plot of $\ln(N_0/N_t)$ vs. time for the experimental conditions, the simple Chick-Watson model was used to describe the inactivation of the spore preparations. The coefficient of dilution (n) was assumed to be equal to unity based upon the fact that data was not extrapolated from specific chlorine and time conditions to other values (Hoff 1986). As a first step in the data analysis, the profile of residual chlorine versus time for each data set (see Table 1) was fitted to a first-order and a second-order decay model. The SAS procedure NLIN was used (SAS Institute 1990) to estimate the model parameters of both models. The scatter plot (Figure 1) of the predicted chlorine values against observed chlorine values clearly shows that the second-order model is a better fit to the chlorine decay data. The fitted curves along with the data for one of the experimental conditions are given in Figure 2. The first-order decay curve with only the initial and final residual chlorine values, where the decay constant k_1 is calculated by Equation (3), is also included in this figure. Figure 2 shows that, for given exposure time t , the first-order decay model with more than two residual chlorine measurements

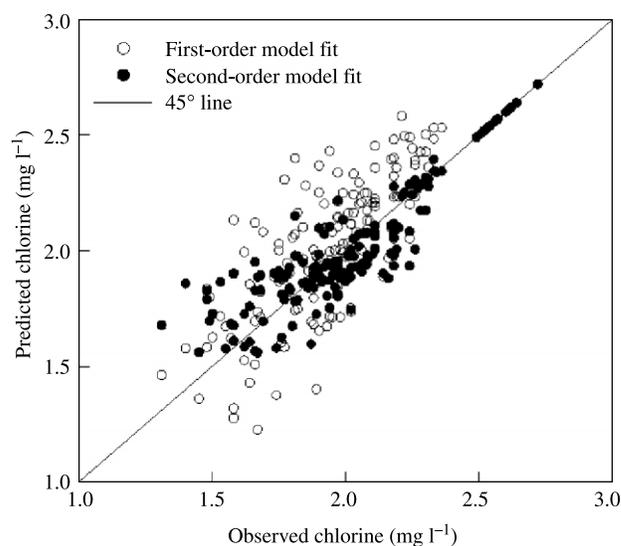


Figure 1 | Comparison of the second-order decay model with the first-order decay model.

gives a smaller Ct (the area under the curve) than the model with only two measurements. The Ct from the second-order model fit is even smaller than the above two Ct values. Thus, the inactivation rate constant K is larger for a second-order decay model fit than for a first-order decay model fit.

As residual chlorine is measured at least twice in between the initial and final exposure time, we can use the second-order decay model to fit the data and then estimate Ct as the area under the fitted curve. As Ct values are estimated, they are subject to estimation error. Treating Ct as a constant will

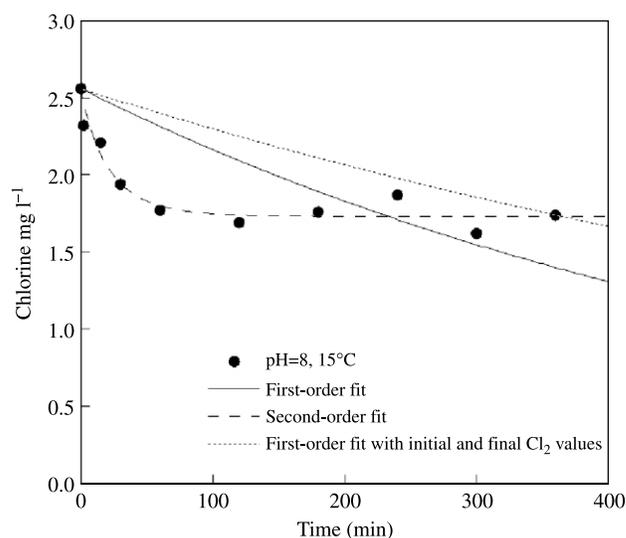


Figure 2 | Comparison of three decay models for one of the free residual chlorine–time profile data sets.

Table 2 | Estimated model parameters for Equation (4)

pH	Temperature (°C)	First-order decay model		Chick-Watson model	
		\hat{k}_1 (Std error)	R^2	\hat{K}_f (Std error)	R^2
7	5	2.596E-3 (3.64E-4)	0.21	8.655E-3 (3.77E-4)	0.94
7	10	4.036E-3 (3.42E-4)	0.68	1.150E-2 (4.36E-4)	0.94
7	15	3.364E-3 (4.38E-4)	0.19	1.234E-2 (5.84E-4)	0.93
7	23	5.071E-3 (7.08E-4)	0.45	4.639E-2 (1.92E-3)	0.95
8	5	6.8E-4 (5.8E-5)	0.57	3.236E-3 (1.26E-4)	0.93
8	10	1.504E-3 (1.80E-4)	0.49	4.254E-3 (2.25E-4)	0.91
8	15	1.722E-3 (1.61E-4)	0.34	4.763E-3 (1.52E-4)	0.97
8	23	3.146E-3 (2.58E-4)	0.68	1.022E-2 (5.97E-4)	0.87

underestimate the inactivation rate constant K of the simple Chick-Watson model. As Ct is estimated from the chlorine decay model and is used in the inactivation model, these models have common model parameters. Thus, as a second step in the data analysis, the above models are fitted simultaneously. To study the effect of chlorine demand on the inactivation kinetics, we also included the first-order decay model for chlorine demand. For given exposure times, t , the first-order chlorine decay and the simple Chick-Watson inactivation models are given by:

$$(C)_t = (C)_0 \cdot e^{-k_1 \cdot t}$$

$$\begin{aligned} \log(N_t/N_0) &= -K_f C_t = -K_f \cdot (C)_0 \cdot \int_0^t e^{-k_1 \cdot x} dx \\ &= -K_f \cdot (C)_0 \cdot [1 - e^{-k_1 \cdot t}]/k_1 \end{aligned} \quad (6)$$

The model parameter k_1 is common to both models and K_f is the inactivation rate constant in Equation (6). Replacing

the first-order decay model in the above equation by the second-order decay model gives:

$$(C)_t = (C)_0 \cdot \frac{(1-r)}{1-r \cdot e^{-k_2 \cdot (1-r) \cdot t}}$$

$$\log(N_t/N_0) = -K_s C_t = -K_s \cdot (C)_0$$

$$\times \int_0^t \frac{(1-r)}{1-r \cdot e^{-k_2 \cdot (1-r) \cdot x}} dx$$

$$= -K_s \cdot (C)_0 \cdot \{(1-r) \cdot t + [1/k_2]$$

$$\times \ln(1 - r \cdot e^{-k_2 \cdot (1-r) \cdot t})/(1-r)\} \quad (7)$$

The inactivation rate constant is denoted by K_s and the common model parameters are k_2 and r ($= 1 - C^*$) in Equation (7). There are three to four data sets for experimental condition (see Table 1) and these are pooled to fit the models described by Equations (6) and (7). The SAS procedure MODEL is used to estimate the model parameters

Table 3 | Estimated model parameters for Equation (5)

pH	Temperature (°C)	Second-order decay model		R^2	Chick-Watson model	
		\hat{k}_2 (Std error)	\hat{r} (Std error)		\hat{K}_i (Std error)	R^2
7	5	6.302E-2 (3.40E-2)	1.907E-1 (2.14E-2)	0.74	9.021E-3 (3.66E-4)	0.95
7	10	1.638E-2 (7.78E-3)	4.418E-1 (1.16E-1)	0.75	1.171E-2 (4.45E-4)	0.94
7	15	3.464E-2 (9.19E-3)	3.312E-1 (2.83E-2)	0.83	1.304E-2 (5.81E-4)	0.92
7	23	9.154E-2 (9.25E-2)	2.067E-1 (4.02E-2)	0.65	4.860E-2 (2.52E-3)	0.94
8	5	1.480E-2 (3.71E-3)	2.388E-1 (9.10E-3)	0.94	3.460E-3 (1.40E-4)	0.92
8	10	2.221E-2 (1.28E-2)	2.439E-1 (3.49E-2)	0.66	4.483E-3 (2.40E-4)	0.92
8	15	2.305E-2 (5.53E-3)	3.839E-1 (1.74E-2)	0.85	5.341E-3 (1.63E-4)	0.96
8	23	4.208E-2 (1.55E-2)	2.289E-1 (2.75E-2)	0.79	1.055E-2 (5.84E-4)	0.89

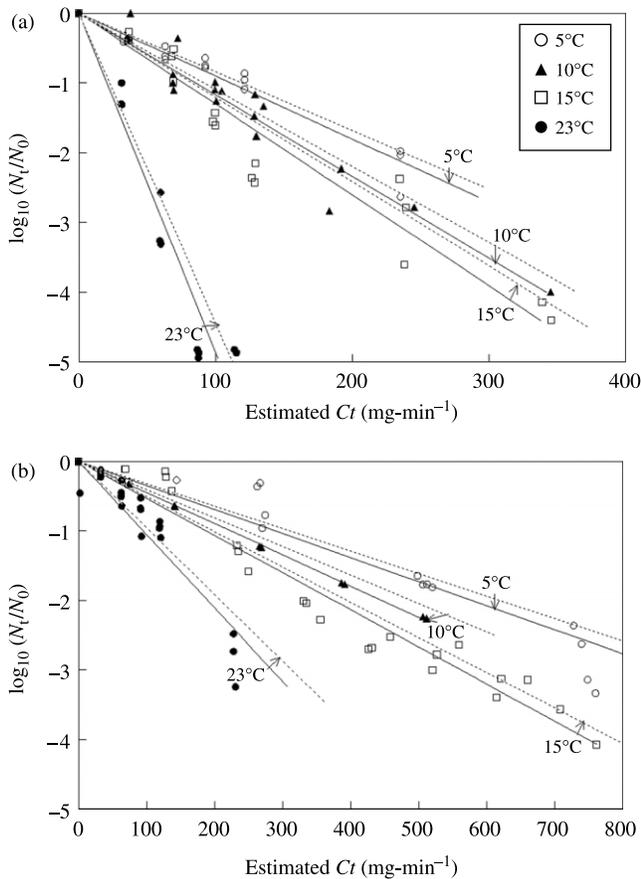


Figure 3 | Inactivation of spores exposed to free chlorine under different experimental conditions: (a) pH 7; (b) pH 8. Solid and dotted lines represent the estimated mean and 95% upper confidence bound Ct values. Symbols: ○, 5°C; ▲, 10°C; □, 15°C; ●, 23°C.

simultaneously. A non-linear two-stage least-squares estimation procedure was used in the ‘Marquardt-Levenberg’ method to estimate these parameters (Marquardt 1963; Amemiya 1974; SAS Institute 1988). It is clear from the

model R^2 (Tables 2 and 3) that a second-order decay model gives a better fit for the chlorine decay data than a first-order fit. As expected the estimate \hat{K}_s of the inactivation rate constant is larger than the corresponding estimate \hat{K}_f for each of the experimental conditions.

For given exposure time t , the area under the fitted second-order decay curve gives an estimate $\hat{C}t$ for Ct and is given by:

$$\hat{C}t = (C)_0 \cdot \left\{ (1 - \hat{r}) \cdot t + \left[\frac{1}{\hat{k}_2} \cdot \ln(1 - \hat{r}) \cdot e^{-\hat{k}_2 \cdot (1 - \hat{r}) \cdot t} \right] / (1 - \hat{r}) \right\} \quad (8)$$

The two-stage least-squares estimates \hat{r} and \hat{k}_2 of the parameters r and k_2 are given in Table 3. The numbers in the parentheses are the estimated standard errors of these parameters. Figure 3(a) and (b) shows the scatter plot of $\log(N_t/N_0)$ against $\hat{C}t$ for different experimental conditions. The plots of mean Ct (solid line) and a corresponding 95% upper confidence bound (dotted line) are also included in the figure. To develop these plots, the distribution of I/K_s , where $I (= \log(N_t/N_0))$ a known inactivation level, should be known. As the second-order decay model of Equation (7) is a non-linear model, we used a simulation method to find the distribution of I/K_s . To iterate samples for this distribution, it is assumed that K_s has a normal distribution, with mean \hat{K}_s and the square of the corresponding estimated standard error as its variance (see Table 3).

The effect of using different chlorine decay models on Ct values for spore inactivation is presented in Table 4. Values were determined using the two point (initial and final) chlorine values in a first-order model and first-order and second-order decay models using intermediate chlorine levels. As expected the two-point first-order model gave the largest Ct values. In some instances there were minimal

Table 4 | Ct ($\text{mg} \cdot \text{min}^{-1}$) values for 3 log spore inactivation using different residual chlorine decay models

pH	Temperature (°C)	Second-order kinetic model	First-order kinetic model	Initial and final Cl_2 model
7	5	333	347	375
7	10	256	261	281
7	15	230	243	261
7	23	62	65	66
8	5	867	927	964
8	10	669	705	718
8	15	562	630	686
8	23	284	294	309

differences between Ct values for the three models. However, under conditions where inactivation occurred at a slower rate (ex. pH 8, 5°C) these differences were more pronounced. Lower Ct values can be an important consideration in utility operations when attempting to balance disinfection by-product formation and adequate disinfection. The use of appropriate models in the development of Ct values is necessary to adequately address these concerns.

CONCLUSIONS

In this study we used experimental data to illustrate the need to incorporate chlorine decay measurements when determining Ct values. Analysis of the data for the inactivation experiments where chlorine levels dissipated during the course of the exposure times indicated that a second-order decay model was most appropriate to model the residual chlorine–time profile. Measuring residual chlorine more than twice during the course of the experiments allowed us to select an appropriate decay model to study the effect of chlorine demand on the inactivation kinetics. These findings are similar to those of Clark (1998) regarding bulk chlorine decay in experiments designed to measure disinfection by-product formation where a second-order model was also determined to be appropriate. As a linear trend was seen in the scatter plot of the log survival ratio–time profile, a simple Chick–Watson kinetic model was used to estimate the inactivation rate constant for the spores.

Traditionally, Ct is treated as a constant in estimating the inactivation rate constant. To account for the error in estimation of Ct , we used a simultaneous modelling approach. Using this approach one can estimate the required Ct which includes an upper bound (or safety factor), instead of calculating a single estimate for Ct .

It is clear from Figure 3(a) and (b) that the inactivation rate constant increases with temperature for both pH levels. This finding suggests that, to fully characterize the inactivation process for a given microorganism, studies should be

conducted at several temperatures and pH levels to determine the effect of these water quality parameters on the inactivation rate constant. The results presented here illustrate the need to make several residual chlorine measurements during the course of the experiments to adequately determine the chlorine decay rate. This determination is crucial for selecting an appropriate model to determine disinfectant concentration to be used in developing Ct tables.

The use of the second-order model yielded lower Ct values than those derived from the mathematically simpler first-order or first-order initial and final models. While the observed differences were not particularly dramatic, generally less than 10%, these lower values may be beneficial in assisting water utilities which need to balance adequate microbial inactivation with the need to control disinfection by-products.

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