# Inactivation of *Bacillus globigii* by chlorination: A hierarchical Bayesian model

M. Sivaganesan, N. J. Adcock and E. W. Rice

## ABSTRACT

Recent events in which spores of *Bacillus anthracis* have been used as a bioterrorist weapon have prompted interest in determining the resistance of this organism to commonly used disinfectants, such as chlorine and ozone. This work was undertaken to study the effect of temperature over the range of 5°C to 30°C for pH levels of 7 or 8, on the inactivation kinetics of the spores of *Bacillus globigii* and to evaluate whether these spores could serve as a surrogate for the spores of *B. anthracis* in chlorine inactivation studies in water. The delayed Chick-Watson model, i.e. a lag phase followed by pseudo-first order rate of inactivation, was found to adequately describe the inactivation kinetics of *B. globigii*. Markov Chain Monte Carlo (MCMC) simulation method was used to estimate the length of the lag phase and the post-lag phase rate constant. As expected, the length of the lag phase decreased with increasing temperature and the post-lag phase rate constant increased with increasing temperature. A hierarchical Bayesian modelling approach was used to model the kinetic parameters of the inactivation model as functions of temperature. The MCMC simulation method was used to estimate the minimum *CT* requirement (with safety factor) for 99% inactivation of *B. globigii*.

**Key words** | lag phase, MCMC method, posterior distribution, rate constant, upper credible bound

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## INTRODUCTION

Recently approved legislation limits the use of the spores of *Bacillus anthracis* to laboratories which have governmental approval for working with select agents. These restrictions have prompted renewed interest in evaluating organisms that might serve as surrogates for the overt pathogen. Information regarding the resistance of *B. anthracis* spores to chlorination is of particular interest in reference to drinking water treatment. There have been limited studies comparing chlorine inactivation of spores of *B. anthracis* with other *Bacillus* species (Fair *et al.* 1947; Brazis *et al.* 1958). These studies were not primarily designed to determine inactivation kinetics, but rather to elucidate basic disinfection concepts such as the ability to measure free available chlorine and the effects of changes in temperature and pH on the inactivation process. More recent studies have concentrated on the use of chlorine as a surface disinfectant (Sagripanti & Bonifacino 1996). The direct comparisons of inactivation data from these reports, as with other comparisons of this sort, are difficult to make owing to differences both in methodology and experimental design.

As little or no inactivation took place during the initial lag phase of the inactivation process, a delayed Chick-Watson model (Sivaganesan *et al.* 2003) was used to model the inactivation data, instead of a simple Chick-Watson model (Hoff 1986). We also compared the delayed Chick-Watson model with 'Hom' model, and found the former to be clearly preferable in our context (see the model development section). Use of a traditional two-step modelling approach fails to take into account the estimation errors in the first step,

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and hence is prone to overstate the accuracy in the final answers. Moreover, the length of the lag phase ($CT_{lag}$) being generally unknown, makes the traditional least squares based approach difficult to implement. Use of the hierarchical Bayesian modelling approach is particularly suitable compared with the least squares based approach, as the former allows us to account for the errors appropriately and is amenable to modelling an unknown length of lag phase. It has been successfully used in modelling in water research as well as in other scientific disciplines (Qian *et al.* 2005; Sivaganesan & Sivaganesan 2005).

Bayesian statistics is based on the idea that the uncertainty of an unknown parameter in the statistical model is described by a probability distribution, known as the posterior distribution. The posterior distribution combines the available prior information expressed in terms of a prior distribution and the information in the sample expressed by the likelihood (joint density), and is proportional to the product of the two. Estimates and confidence intervals for the model parameters are obtained from the posterior distribution. Closed form solutions for these quantities are usually not available, and this is where the MCMC methods (Gelman *et al.* 1995; Brooks 1998) are used to compute the desired estimates.

## MATERIALS AND METHODS

In this study the sporicidal effectiveness of free chlorine was determined at two pH levels and temperatures over the range of 5°C to 30°C. Table 1 gives the experimental conditions. These inactivation experiments were conducted under chlorine demand-free conditions. For a given experimental condition, concentrations of chlorine in mg $l^{-1}$ were measured at different times (in minutes) over a time period $t_0$. First these concentrations were used to estimate the exponential decay constant $k$. Then for given t ($\leq t_0$) in minutes, the corresponding $CT$ value was calculated as the area under the exponential decay curve and is given by:

$$CT = Cl_0 \cdot \int_0^t e^{-kx} dx \qquad (1)$$

where $Cl_0$ is the initial residual chlorine concentration in mg $l^{-1}$. For each of the experimental conditions and exposure time, t, corresponding $CT$ values were calculated using Equation (1).

*Bacillus globigii* was obtained from Dr John Wright, US Army Dugway Proving Ground. Endospores were produced in broth culture using a generic sporulation medium (Coroller *et al.* 2001). The cultures were grown at 35°C with agitation on a rotary shaker for five days. Spores were purified by gradient separation using RenoCal-76™ (Bracco Diagnostics, Princeton, New Jersey) and washed three times by centrifugation with sterile deionized water as previously described (Nicholson & Setlow 1990). Purified spore preparations were examined using phase contrast microscopy and stored in 40% (v/v) ethanol at 5°C until the time of use.

Chlorine demand free (CDF) buffer (0.05 M $KH_2PO_4$) was used in the experiments. The buffer was made chlorine demand free by adding reagent grade sodium hypochlorite (4–6%) to the buffer to achieve a free chlorine residual of approximately 3.0 mg $l^{-1}$. The pH of the buffer was adjusted by the addition of 10 M sodium hydroxide. The buffer was boiled for 5 minutes and transferred to 4 l beakers and exposed to short wave ultraviolet (UV) irradiation in a biological safety cabinet for 48 hours to remove the chlorine. The buffer was then sterilized by autoclaving and stored in a sealed container for no longer than 3 weeks. An appropriate volume of a 1:200 (V/V) dilution sodium hypochlorite solution was added to the CDF buffer to obtain the desired free chlorine level.

Except for the ambient room temperature, the inactivation experiments were conducted in a re-circulating temperature controlled water bath. All temperatures in the reaction vessels were verified using a mercury filled glass thermometer. Borosilicate glass beakers (1,000 ml) containing 500 ml of buffer served as the reaction vessels. Reaction vessels were continuously stirred using a magnetic stirring apparatus. A submersible stirring apparatus was used for the experiments conducted at a re-circulating temperature controlled water bath. The vessels were inoculated with the various spore preparations to yield an initial level of approximately $1 \times 10^4$ CFU $ml^{-1}$. Chlorine concentrations were determined at each exposure time using the *N,N*-diethyl-*p*-phenylenediamine (DPD) colorimetric method (*Standard Methods* 1998). Controls for these experiments consisted of CDF buffer without a chlorine residual. Samples were withdrawn from the reaction vessels at the

**Table 1** | Summary of experimental conditions corresponding to datasets for the inactivation of spores of *B. globigii* with chlorine

| Dataset | pH | T (°C) | $-\text{Log}\ (N_t/N_0)$ | $Cl_0$ (mg $l^{-1}$) | *CT* (mg min $l^{-1}$) | Sample size (*n*) |
|---|---|---|---|---|---|---|
| 1*, 2, 3 | 7 | 5 | 0–4.397 | 2.1 | 0–615 | 9, 9, 9 |
| 4 * | 7 | 8 | 0–5.059 | 2.1 | 0–494 | 9 |
| 5 | 7 | 10 | 0–3.966 | 2.2 | 0–359 | 7 |
| 6 | 7 | 15 | 0–4.823 | 2.1 | 0–307 | 8 |
| 7 | 7 | 17 | 0–4.431 | 2.2 | 0–297 | 11 |
| 8–10, 11* | 7 | 23 | 0–4.589 | 2.2 | 0–180 | 6, 7, 6, 5 |
| 12 | 7 | 30 | 0–4.228 | 2.1 | 0–120 | 5 |
| 13–14, 15* | 8 | 5 | 0–3.354 | 2.1 | 0–1294 | 11, 11, 11 |
| 16 | 8 | 8 | 0–4.712 | 2.0 | 0–1254 | 8 |
| 17 | 8 | 10 | 0–4.843 | 1.1 | 0–1067 | 10 |
| 18 * | 8 | 15 | 0–3.847 | 1.1 | 0–821 | 8 |
| 19 | 8 | 17 | 0–4.692 | 2.1 | 0–719 | 9 |
| 20*, 21–23 | 8 | 23 | 0–4.879 | 2.1(2), 3.1(2) | 0–592 | 10, 8, 9, 7 |
| 24 | 8 | 30 | 0–4.861 | 3.1 | 0–410 | 8 |

*used to validate the general model

various exposure times and any residual chlorine was immediately neutralized by the addition of 0.1 ml of a 10% (W/V) sodium thiosulphate solution. Control and test samples were treated in the same manner throughout the experiments. The number of spores present in the control and chlorine exposed samples were determined by cultural count using the membrane filtration procedure as previously described (Rice *et al.* 1996).

## MODEL DEVELOPMENT

### Delayed Chick-Watson model

For each of the experimental conditions given in Table 1, the plot of $\log_{10}$ ratio of survivors against *CT* clearly showed a lag phase (or shoulder) followed by a linear decay curve. Thus, instead of a simple Chick-Watson kinetic model (Hoff 1986), a delayed Chick-Watson kinetic model was used to model inactivation curves. The two inactivation kinetic parameters, length of lag phase ($CT_{\text{lag}}$) and the decay rate constant $\beta$ are estimated via the Bayesian statistical methods described in Sivaganesan *et al.* (2003) for each of the experimental conditions. For a given dataset, let $n$ be the number of data points. The delayed Chick-Watson model is given by:

$$Y_i = \log(N_t/N_0)_i = \mu_i + \varepsilon_i \quad i = 1, \ldots, n$$

where,

$$\mu_i = 0 \qquad \text{if}(CT)_i < CT_{\text{lag}}$$

$$\mu_i = -\beta[(CT)_i - CT_{\text{lag}}] \quad \text{if}\ (CT)_i > CT_{\text{lag}} \tag{2}$$

The response variable, $Y_i$, in the above model is the survival ratio $(N_t/N_0)_i$ (in log base 10 units) and it is written as a function of the lag-phase, $CT_{\text{lag}}$, and the post-shoulder

first-order inactivation rate constant $\beta$. The random error terms $\varepsilon_i$ in Equation (2) are assumed to be normally distributed with mean 0 and variance $\sigma^2$. As no prior information is assumed for the rate constant $\beta$ we use a diffuse normal prior with mean 0 and variance $10^6$. We also use a diffuse Inverse-Gamma (0.001, 0.001) prior for $\sigma^2$, which is an approximation to the commonly used Jeffrey's prior $\text{pr}(\sigma^2) \propto 1/\sigma^2$. As $CT_{\text{lag}}$ could be anywhere in the range of the $CT$ values of a given dataset, a uniform prior distribution is assumed for $CT_{\text{lag}}$ between 0 and the maximum $CT$ value, $CT_{\max}$, of the dataset. These priors can be written as:

$$\beta \sim N(0, 10^6)$$

$$\sigma^2 \sim \text{Inverse} - \text{Gamma}\ (0.001, 0.001)$$

$$CT_{\text{lag}} \sim \text{Uniform}(0, CT_{\max}) \qquad (3)$$

According to Bayes theorem, the posterior distribution of the model parameters $\beta$, $CT_{\text{lag}}$ and $\sigma^2$ given the data $y_1, \ldots, y_n$ is proportional to the product of the normal densities (or likelihood) of all $Y_i$ values evaluated at $y_1, \ldots, y_n$ (given $\mu_i, \sigma^2$) and prior distributions given by Equation (3). Thus, the posterior distributions of the three parameters $k$, $CT_{\text{lag}}$ and $\sigma^2$ are determined by data, model and prior distribution of the parameters. The MCMC simulation method is used to estimate these parameters for each of the datasets given in Table 1. The MCMC calculations were done using the software WinBUGS (www.mrc-bsu.cam.ac.uk/bugs).

Estimating $CT$ for a given log inactivation $\log(N_t/N_0)$ $(= Y_0)$ is a typical calibration problem. This can be easily solved by generating the posterior distribution of $CT$ values. Equation (2) can be rearranged to get the following expression giving $CT$ as a function of $\beta$ and $CT_{\text{lag}}$:

$$CT = -[(Y_0 - \varepsilon)/\beta] + CT_{\text{lag}} \quad \text{if } \log(N_t/N_0) > 0 \qquad (4)$$

Note that there is no solution for $CT$ if $\log(N_t/N_0) > 0$. Thus by requesting the posterior distribution of $CT$ values from the WINBUGS program, one can construct a confidence interval for $CT$ given a single future value of $\log(N_t/N_0)$. Constructing a confidence interval for $CT$ given the mean value of $\log(N_t/N_0)$ does not include $\varepsilon$ in Equation (4). For the purpose of illustration, a scatter plot of the second dataset (see Table 1) is shown in Figure 1 together with the corresponding fitted inactivation curve, the 95% upper credible bound (UCB) for $CT$ given a mean log inactivation, and the 95%UCB for $CT$ given a single log inactivation value. The WinBUGS code to generate the statistics such as mean, standard deviation, median, and 90% upper confidence limit for the model parameters and predicted $CT$ values are given in the Appendix A for the same dataset. The statistics are based on the posterior distributions of the parameters. Fitted inactivation curves for all the experimental conditions at pH 7 are given in Figure 2. Corresponding curves at pH 8 are given in Figure 3. As there is more than one dataset for 5°C and for 23°C, the datasets are pooled at each of these two temperatures to obtain the corresponding fitted curves.

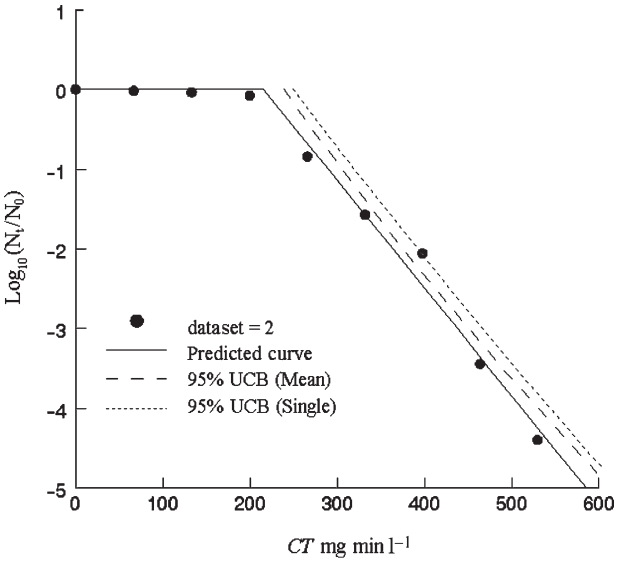


**Figure 1** | Fitted curve and 95% upper credible bound (UCB) for $CT$ for one of the datasets (see Table 1).

The shoulder effect of an inactivation curve can also be accounted by an empirical coefficient 'm' in the 'Hom' model. To compare the previously described delayed Chick-Watson model with the Hom model, the error sum of squares are calculated for each of the experimental conditions given in Table 1. More than half the error sum of squares are smaller for the delayed Chick-Watson model than the Hom model. The mean squared errors are 0.0329 and 0.0366, respectively, for these models. Moreover, when using the Hom model, none of the scatter plots of the estimated model parameters against temperature showed any relationship for either pH value, whereas the corresponding plots of the delayed Chick-Watson model showed a clear relationship for both pH

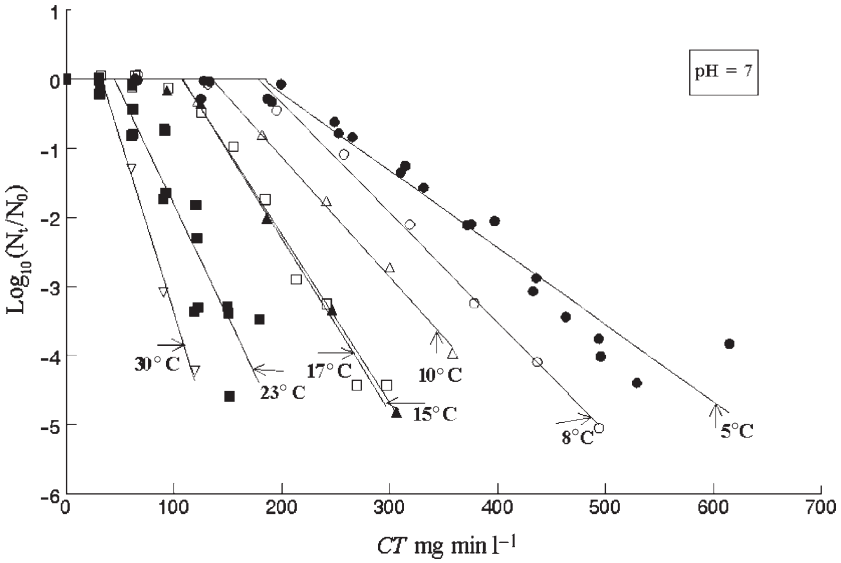


**Figure 2** | Fitted delayed Chick-Watson model curves for the datasets 1 to 12.

values. Thus, we find that the simpler delayed Chick-Watson model (a piece-wise linear model with one change point) provides a better fit, and gives more sensible results than the Hom model. Thus in this context the use of the delayed Chick-Watson is preferable.

## Hierarchical Bayesian delayed Chick-Watson model

As expected the (posterior) mean of the rate constant $\beta$ increases with temperature T. Inactivation also occurred more rapidly as anticipated at the lower pH level (see Figure 4). These results are consistent with the well-established observations regarding the role of temperature and pH in chlorine inactivation studies (Hoff 1986). Moreover, the estimate of length of the shoulder $CT_{lag}$ (i.e. its posterior mean) decreases as the temperature increases. The length of the shoulder is at least one log unit (base e) larger at pH 8 than at pH 7 for any temperature between 5°C and 30°C (see Figure 5). As depicted in Figures 4 and 5, the estimated posterior mean $k$

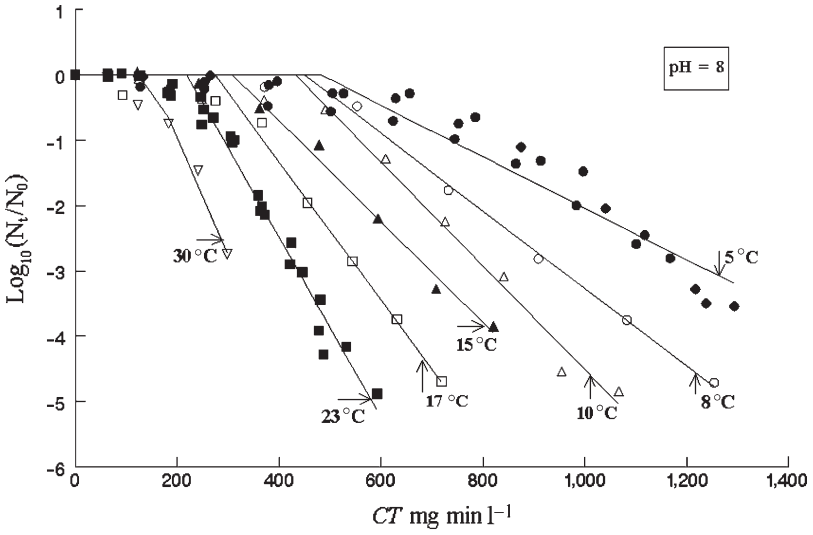


**Figure 3** | Fitted delayed Chick-Watson model curves for the datasets 13 to 24.

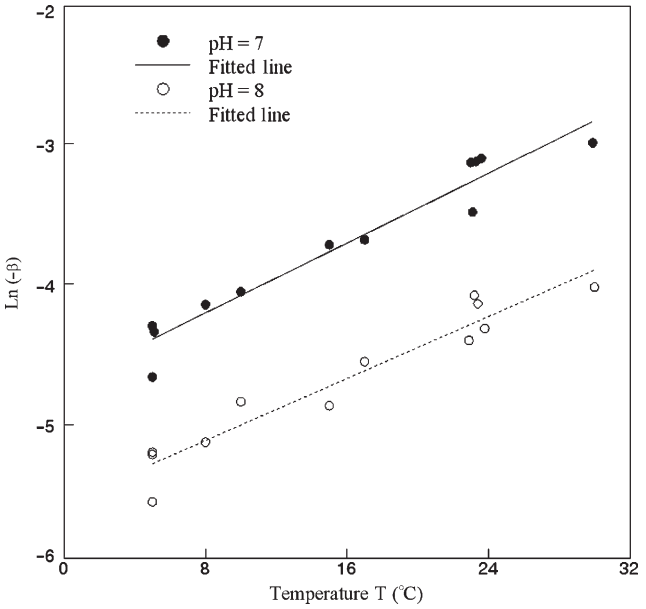


**Figure 4** | Plots of estimated rate constant against temperature along with the fitted lines.

and $CT_{lag}$ values were found to have log-linear relationships with temperature.

Traditionally, model fitting is done using a two-step approach to study the effect of temperature on decay rate $\beta$. First, for each of the datasets, the least squares method is used to estimate $\beta$. The estimated values of $\beta$ are then related to the corresponding temperature values via a regression model. In doing so, this approach implicitly treats the estimates $\beta$ as 'observed' values while they are only estimates of the unknown rate parameters, and ignores the error in such estimation. Accounting for these errors is important especially when they are heterogeneous (i.e. they vary with the temperature). Moreover the two-step approach could not easily allow for the presence of a shoulder. When the modelling is done using the two-step procedure as described above, it is difficult to correctly account for the estimation errors in the first step. As such, these errors are basically ignored, leading to an underestimation of the reported errors in the final answers.

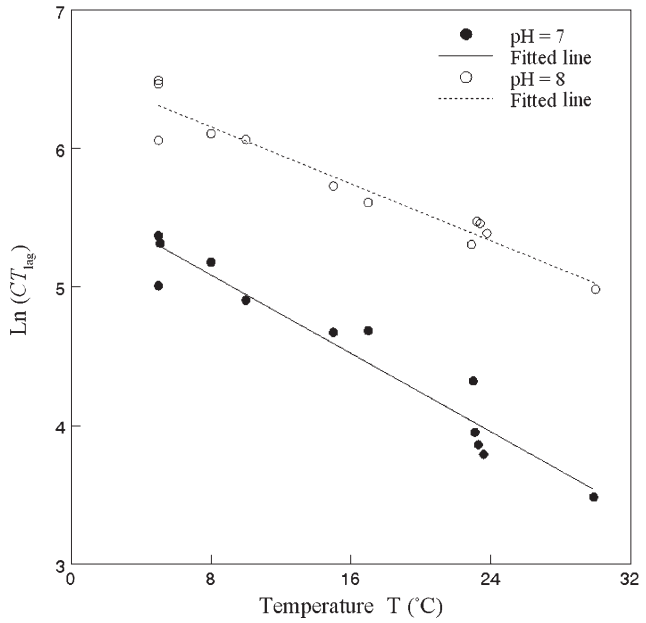


**Figure 5** | Plots of estimated $CT_{lag}$ against temperature along with the fitted lines.

One of the goals of this paper is to appropriately account for these errors by simultaneously fitting the models in the two steps. The use of a hierarchical Bayesian modelling approach is particularly suitable for this purpose. It allows for the simultaneous modelling of the two steps, and hence correctly accounts for the errors in both steps according to the rules of probability. This modelling is increasingly used in many scientific disciplines; examples of its use in water research can be found in Qian *et al.* (2005) and Sivaganesan & Sivaganesan (2005). We used a hierarchical Bayesian model (see Equation (5) below) to simultaneously fit the delayed Chick-Watson model, and the model relating the rate constant to temperature. The prior distributions used here are the commonly used non-informative priors; these are meant to allow the likelihood function of the observed data to be the primary source of information in determining the final inference. Since closed form solutions for estimates based on hierarchical Bayesian models are usually not available, as is also the case here, the MCMC simulation method is commonly used to fit such models. For a review of MCMC methods see Gilks *et al.* (1996). The MCMC method required to fit our model can be easily implemented using the publicly available WinBUGS software.

As there are only two pH levels, the modelling was performed separately for the two pH levels. Three datasets (see Table 1) were set aside at each pH level to verify the proposed hierarchical model. As there was more than one dataset at 5°C and 23°C, one dataset was randomly selected from each of these temperatures and the third dataset was randomly selected from the remaining temperatures.

For a given number of datasets (or experimental conditions), $m$, the hierarchical Bayesian delayed Chick-Watson model is given by:

$$Y_{ij} = \log(N_t/N_0)_{ij} = \mu_{ij} + \varepsilon_{ij} \quad i = 1, \ldots, n_j;\ j = 1, \ldots, m$$

where,

$$\mu_{ij} = 0 \qquad \text{if } (CT)_{ij} > CT_{lag(j)}$$

$$= -\beta_j[(CT)_{ij} - CT_{lag(j)}] \quad \text{if } (CT)_{ij} > CT_{lag(j)}$$

$$\ln(\beta_j) = a + b \cdot T_j + e_{1j}$$

$$\ln(CT_{lag(j)}) = c + d \cdot T_j + e_{2j} \tag{5}$$

In Equation (5), $Y_{ij}$ is the log-inactivation ratio for the $i$th dose of the $j$th dataset. The corresponding random error term $\varepsilon_{ij}$ is assumed to have a normal distribution $N(0, \sigma_j^2)$. Similarly for the $j$th dataset, the random error terms $e_{1j}$ and $e_{2j}$ (for the two log-linear relationships with temperature) are assumed to have $N(0, \sigma_{e1}^2)$ and $N(0, \sigma_{e2}^2)$, respectively. The rate constant and the length of the shoulder for the $j$th dataset are denoted by $\beta_j$ and $CT_{lag(j)}$. For the *B. globigii* study data, the MCMC simulation method is employed using the WinBUGS software to obtain the posterior distributions of all the model parameters. The WinBUGS code for the hierarchical Bayesian delayed Chick-Watson model is given in Appendix B.

As no prior information is available about any of the seven model parameters, diffuse normal priors with mean 0 and variance $10^6$ are used for the intercept parameters $a$, $c$ and the slope parameters $b$, $d$. To study the effect of these priors, the variance parameters were increased. No appreciable difference was seen in the posterior distribution of any of these four parameters for larger values of the variance parameter. We used a diffuse inverse-gamma (0.001, 0.001) prior for each of the three variance parameters $\sigma_{e1}^2$, $\sigma_{e2}^2$ and $\sigma_j^2$, which is an approximation to the commonly used Jeffrey's prior $\text{pr}(\sigma^2) \propto 1/\sigma^2$. Smaller values for the parameters of the inverse-gamma prior did not influence the estimates of the model parameters. The estimated posterior mean, median and the 95% credible intervals for a, b, c, d, $\sigma_{e1}^2$, $\sigma_{e2}^2$ and $\sigma_j^2$, are given in Table 2. Notice that the range is reported for the random error variance ${\sigma_j}^2$.

**Table 2** | Estimated posterior mean, median and 95% credible interval for the model parameters given in Equation (5)

| pH | Parameter | Mean | Median | 95% Credible interval |
|---|---|---|---|---|
| 7 | a | −4.57 | −4.57 | (−4.78, −4.33) |
| | b | 0.05 | 0.05 | (0.04, 0.06) |
| | c | 5.81 | 5.80 | (5.57, 6.14) |
| | d | −0.08 | −0.08 | (−0.09, −0.06) |
| | $\sigma_j^2$ * | 0.012–0.176 | 0.008–0.131 | (0.003, 0.578) |
| | $\sigma_{e1}^2$ | 0.020 | 0.015 | (0.004, 0.068) |
| | $\sigma_{e2}^2$ | 0.029 | 0.022 | (0.005, 0.101) |
| 8 | A | −5.58 | −5.59 | (−5.84, −5.33) |
| | B | 0.06 | 0.06 | (0.04, 0.07) |
| | C | 6.53 | 6.54 | (6.31, 6.71) |
| | D | −0.05 | −0.05 | (−0.06, −0.04) |
| | $\sigma_j^2$ * | 0.024–0.097 | 0.017–0.082 | (0.006, 0.242) |
| | $\sigma_{e1}^2$ | 0.022 | 0.017 | (0.004, 0.076) |
| | $\sigma_{e2}^2$ | 0.012 | 0.009 | (0.003, 0.040) |

*estimated range for the random error variance

## Model validation

The log-inactivation ratios are estimated for each of the six datasets that were previously set aside for verification purposes. For given temperature T and $CT$, the mean log-inactivation ratio is estimated as:

$$\log(N_t/N_0) = -\exp(a + b \cdot T)[CT - \exp(c + d \cdot T)]$$

$$\text{if } CT > \exp(c + d \cdot T) \tag{6}$$

$$= 0 \text{ otherwise}$$

where, the estimated posterior mean values of a, b, c and d are given in Table 2. The results from Equation (6) are compared against the corresponding log-inactivation ratios of the previously set aside datasets in Figure 6. It can be seen that the model predictions are in good agreement with the data.

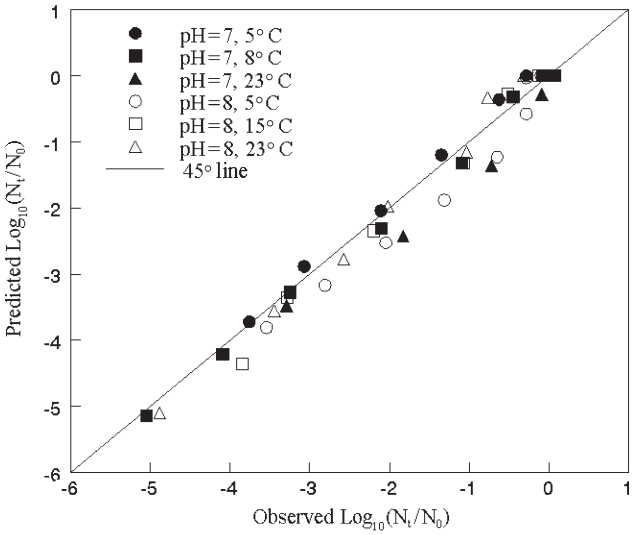


**Figure 6** | Comparison of predicted values from Equation (6) against observed values for selected datasets.

## ESTIMATING *CT* FOR GIVEN TEMPERATURE AND INACTIVATION LEVEL

Estimating mean *CT* for *B. globigii* spp. corresponding to a given mean log-inactivation ratio $I$ and temperature $T_0$ is the main goal of the inactivation study described in this paper. This can be easily obtained by simulating the posterior distribution of *CT*. To do this, first we write *CT* as a function of the model parameters given by Equation (4). The functional relationship is given by:

$$CT = [I/\exp(a + b \cdot T_0)] + \exp(c + d \cdot T_0) \quad (7)$$

Then MCMC samples for *CT* by using the posterior distributions of the parameters $a$, $b$, $c$ and $d$ are generated. The estimated posterior mean *CT* values (mg-min $l^{-1}$) for one, two and three orders of magnitude inactivation $I$ of the spores of *B. globigii* spp. are given in Table 3. To compare the resistance of *B. globigii* spp. with other surrogates, the mean *CT* values of *B. anthracis* Sterne, *B. cereus*, *B. thuringiensis* (Rice *et al.* 2005) and the virulent *B. anthracis* Ames strain (Rose *et al.* 2005) are shown in Table 3. The required mean *CT* values for *B. globigii* are relatively higher than the corresponding mean *CT* of the three surrogates and the virulent strain. Thus, this study shows that spores of *B. globigii* are more resistant than the spores of other *Bacillus* spp. studied by Rice *et al.* (2005) and Rose *et al.* (2005). The required 95% upper credible bound *CT* values for *B. globigii* are given in Figure 7 for temperatures between 5 and 30°C.

**Table 3** | Mean *CT* values for inactivation of spores of *Bacillus* spp

| | | | *CT* (mg-min $l^{-1}$) | | | | |
|---|---|---|---|---|---|---|---|
| **Temp °C** | **pH** | **$Log_{10}$ inactivation** | ***B. globigii*** | ***B. anthracis* Ames**[a] | ***B. anthracis* Sterne**[b] | ***B. cereus***[b] | ***B. thuringiensis***[b] |
| 23 | 7 | 2 | 108 | 79 | 45 | 41 | 66 |
| | | 3 | 136 | 102 | 68 | 62 | 99 |
| | 8 | 2 | 367 | ND[c] | 127 | 132 | 246 |
| | | 3 | 438 | ND | 191 | 199 | 369 |
| 5 | 7 | 2 | 372 | 220 | 140 | 117 | 229 |
| | | 3 | 446 | 339 | 210 | 175 | 344 |
| | 8 | 2 | 943 | ND | 319 | 340 | 481 |
| | | 3 | 1144 | ND | 478 | 510 | 721 |

[a]Rose *et al.* (2005)
[b]Rice *et al.* (2005)
[c]Not determined

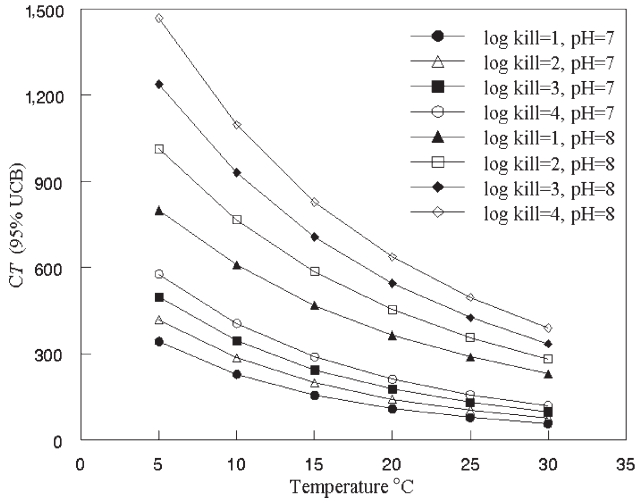


**Figure 7** | Estimated 95% upper credible bound (UCB) for *CT* at different temperatures.

## CONCLUSIONS

There are various sources of errors in chlorine inactivation studies, which, we have shown, can be appropriately accounted for by simultaneously modelling the delayed Chick-Watson model and the two regression models (Equation 5), within a Bayesian hierarchical framework. Monte Carlo samples from the posterior distributions were used to estimate the model parameters and to estimate the minimum *CT* requirement for a given temperature and a percentage inactivation of *B. globigii* spores. Thus, by using this approach, one can estimate an upper bound for the minimum required *CT*.

The model developed in this paper can be used to find confidence intervals for the lag phase *CT*, rate constant $\beta$, and minimum *CT* requirement corresponding to a given temperature and target survival ratio of *B. globigii* spores. This model presents a single step approach for water utilities and regulatory agencies to decide on the level of safety needed when developing treatment requirements for the inactivation of *Bacillus* spores. Future studies directly comparing the inactivation of spores of other surrogate species with spores of the select agent would be beneficial in evaluating the use of surrogate *Bacillus* spp. as an alternative to *B. anthracis* in disinfection studies.

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## APPENDIX A

### Example

The data used in Figure 1 are used here as an example to illustrate the Bayesian method of estimating the delayed Chick-Watson regression model parameters.

Program code for WINBUGS software:

```
model
{
   for(i in 1: n){
     Y[i] ~ dnorm(mu[i],tau)
   mu[i] <- -beta * (CT[i] - CTlag) * step(CT[i]-CTlag) }
# predict CT for given mean log inactivation and single
   log inactivation.

for(i in 1:M){
   CT[i] <- -((Yp[i])/beta) + CTlag
   CT1[i] <- -((Yp[i]-e[i])/beta) + CTlag
   e[i] ~ dnorm(0,tau) }

# non_informative priors (variance σ^2 = 1/τ)
   tau ~ dgamma(.001,.001)
   beta ~ dnorm(0.0,1.0E-6)I(0,)
   CTlag ~ dunif(0,400)
   sigma^2 <- -1/ tau
}

# initial values
list(tau = 1, beta = .008, CTlag = 200,
   ε = c(0,0,0,0,0,0,0,0,0,0,0))

# data
list(n = 9, M = 11,max = 400, Yp = c(0, −.5, − 1,
    − 1.5, − 2, − 2.5, − 3, − 3.5, − 4, − 4.5, − 5))
```

| Y[] | CT[] |
|---|---|
| 0 | 0 |
| −0.01773 | 66.538 |
| −0.03621 | 132.952 |
| … | |
| … | |
| END; | |

### Results (from WinBUGS)

| Parameter | Mean | Std dev. | Median | 95% CI |
|---|---|---|---|---|
| $\beta$ | 0.0136 | 1.057E-3 | 0.0136 | 0.0153 |
| $\sigma^2$ | 0.0469 | 0.0391 | 0.0367 | 0.1086 |
| $CT_{lag}$ | 215.2 | 15.83 | 216.0 | 238.8 |

| $\log_{10}(N/N_0)$ | Parameter | Mean | Std dev. | Median | 95% CI (mean) | 95% CI (single) |
|---|---|---|---|---|---|---|
| 0.0 | *CT*[1] | 215.2 | 15.83 | 216.0 | 238.8 | 249.7 |
| −0.5 | *CT*[2] | 252.2 | 13.32 | 252.8 | 72.2 | 84.5 |
| −1.0 | *T* [3] | 289.2 | 11.00 | 289.6 | 305.9 | 319.9 |
| −1.5 | *CT* [4] | 326.2 | 9.01 | 326.4 | 340.1 | 355.1 |
| −2.0 | *CT* [5] | 363.2 | 7.623 | 363.3 | 375.2 | 391.9 |
| −2.5 | *CT* [6] | 400.2 | 7.193 | 400.1 | 411.9 | 428.0 |
| −3.0 | *CT* [7] | 437.2 | 7.879 | 436.9 | 450.3 | 466.6 |
| −3.5 | *CT* [8] | 474.2 | 9.44 | 473.7 | 490.0 | 504.6 |
| −4.0 | *CT* [9] | 511.2 | 11.53 | 510.5 | 530.5 | 544.1 |
| −4.5 | *CT* [10] | 548.2 | 13.9 | 547.2 | 571.6 | 583.4 |
| −5.0 | *CT* [11] | 585.2 | 16.45 | 584.1 | 613.1 | 623.3 |

Notice that, for a given value of $Y$ ( $= \log(N/N_0)$) the corresponding *CT* value is estimated using the posterior distribution of *CT* given by Equation (4). The summary statistics (mean, standard deviation, median and 95% upper confidence limits) correspond to the posterior distribution (with 25,000 iterations) of the parameters. These values are used to create the fitted and 95% upper confidence limit curves in Figure 1. The estimates for *CT*[5] and CT[7] correspond to 90% and 99% inactivation of the oocysts.

## APPENDIX B

### WinBUGS program code for Equation (5)

```
model
     {
     for(i in 1: n) {
       Y[i] ~ dnorm(mu[i],tau[set[i]])
```

```
    mu [i] <- - beta[set[i]] * (CT[i] - CT_lag[set[i]])
      * step(CT[i]-CT_lag[set[i]]) }
    for (i in 1: m) {
    beta[i] <- exp(a + b * T[i] + e1[i])
    CT_lag[i] <- exp(c + d * T[i] + e[i])
    e1[i] ~ dnorm(0, tau1)
    e2[i] ~ dnorm(0, tau2)}
# non_informative priors
    for (i in 1:m) {
    tau[i] ~ dgamma(.0001,.0001)
    sigma^2[i] <- 1/tau[i] }
    a ~ dnorm(0,1.0E-4)
    b ~ dnorm(0,1.E-4)I(0,)
    c ~ dnorm(0,1.0E-4)
    d ~ dnorm(0,1.E-4)I(,0)
    tau1 ~ dgamma(0.001,0.001)
    tau2 ~ dgamma(0.001,0.001)
    sigma^21 <- 1/tau1
    sigma^22 <- 1/tau2
    }

  # initials for pH = 7
list(a = -5.6, b = .06, c = 3.01, d = -.05, tau1 = 7.3,
  tau2 = 5, tau = c(2,2,2,2,2,2,2,2,2,2,2,2))

# data

list(m = 12, n = 92)
...
```

| *Y*[] | *CT*[] | T [] | set[] |
|---|---|---|---|
| 0 | 0 | 5 | 1 |
| −0.03342 | 62.577 | 5 | 1 |
| −0.28524 | 124.91 | 5 | 1 |
| ... | | | |
| 0 | 0 | 5 | 2 |
| −0.01773 | 66.538 | 5 | 2 |
| −0.03621 | 132.952 | 5 | 2 |
| ... | | | |

```
END;
```

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