USEPA Research on Monochloramine Disinfection Kinetics of *Nitrosomonas europaea*

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Background

Based on utility surveys, 30 to 63% of utilities practicing chloramination for secondary disinfection experience nitrification episodes (American Water Works Association 2006). Nitrification in drinking water distribution systems is undesirable and may result in water quality degradation (e.g., disinfectant depletion, coliform occurrences, or nitrite/nitrate formation) and subsequent non-compliance with existing regulations (e.g., Surface Water Treatment Rule or Total Coliform Rule).

As a first step toward gaining better information on ammonia-oxidizing bacteria (AOB) disinfection in chloraminated drinking water distribution systems, a culture-independent method with future applicability to mixed-culture AOB was implemented with *Nitrosomonas europaea*. The culture-independent method combines propidium monoazide (PMA), which selectively removes DNA from membrane-compromised cells and/or inhibits its amplification by PCR (Nocker et al. 2007), with a quantitative PCR (qPCR) method (Figure 1) developed for detection of AOB in chloraminated drinking water distribution systems (Regan et al. 2007). The results using PMA-qPCR were compared with those obtained using another culture-independent membrane integrity based technique, LIVE/DEAD® BacLight™ (LD) (Figure 2), that was previously used to determine *N. europaea* monochloramine disinfection kinetics (Oldenburg et al. 2002).

Methods

Both methods were first verified with mixtures of heat-killed (nonviable) and non-heat-killed (viable) cells before conducting a series of batch disinfection experiments with stationary phase cultures (batch grown seven days) at pH 8.0; 10 mM phosphate buffered saline (PBS, 10 mM Na_2HPO_4, 130 mM NaCl); 25°C; and 5, 10, and 20 mg Cl_2/L monochloramine. Further experiments were conducted in additional phosphate buffers (1 mM, 10 mM, and 50 mM Na_2HPO_4) at pH 8.0; 25°C; and 5 mg Cl_2/L monochloramine. Kinetic parameters were estimated for the Delayed Chick-Watson disinfection model (Equation 1), accounting for an initial lag phase where no disinfection occurs followed by a pseudo-first order phase.

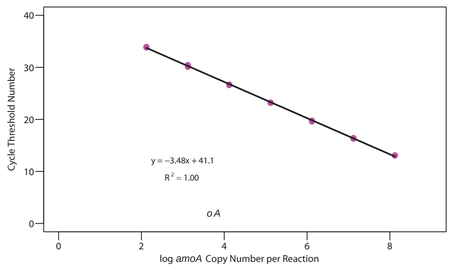

Figure 1. Example qPCR standard curve for *amoA*.

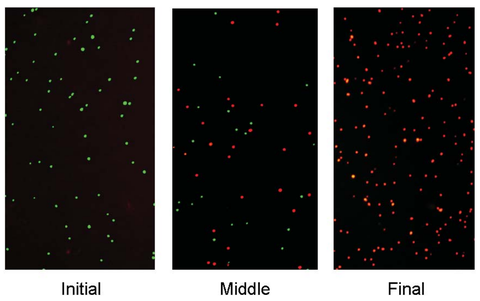

Figure 2. Example LIVE/DEAD images for batch disinfection experiments showing an initial image (time = 0), middle image (time > 0), and final image (time = final).

Equation 1

$$\ln \frac{N/N_T}{N_0/N_T} = \ln \frac{N}{N_0} = \begin{cases} 0 & \text{for } Ct \le b \\ -k(Ct-b) & \text{for } Ct > b \end{cases}$$

$$\ln N = \begin{cases} \ln N_0 & \text{for } Ct \le b \\ \ln N_0 - k(Ct-b) & \text{for } Ct > b \end{cases}$$

C = disinfectant concentration [mg Cl_2/L]
t = time [min]
k = disinfectant rate constant [L/mg Cl_2-min]
b = lag coefficient [mg Cl_2-min/L]
N = viable bacteria at t [cells]
N_0 = initial viable bacteria at t=0 [cells]
N/N_T = viable bacteria ratio at t [-]
N_0/N_T = initial viable bacteria ratio at t=0 [-]

Results

Control experiments: Heat-killed control experiments (Figure 3) verified that both methods are able to selectively measure viable cells in a mixture of *N. europaea* viable and nonviable cells.

PBS experiments: The Delayed Chick-Watson model was implemented in WinBUGS (Bayesian analysis software) to estimate model parameters and their 95% credible bounds (Figure 4). Figure 5 displays the joint 95% highest posterior density (HPD) regions and samples from the kinetic parameter posterior distributions. The areas in Figure 5 highlight the greater uncertainty in the estimate of *b* with PMA-qPCR and the difference in *k* between the two methods. LD and PMA-qPCR resulted in similar but significantly different estimates of the disinfection kinetic parameters.

Various buffer experiments: To evaluate the buffer choice on the disinfection kinetics, further experiments were conducted with various phosphate buffers. Figure 6 summarizes the resulting kinetic parameter 95% HPD regions. The buffer used showed a significant effect on the LD and PMA-qPCR estimated kinetic parameters.

Experiment Summary: Table 1 summarizes the results for the estimated kinetic parameters (*b* and *k*) for all experiments. For comparison purposes, Oldenburg et al. (2002) data are included.

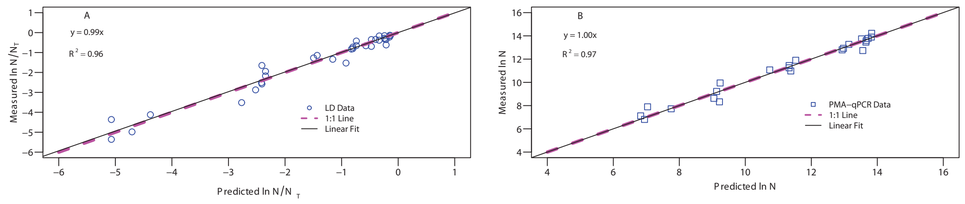

Figure 3. Heat-killed (15 minutes at 72°C) control experimental data for (A) LD and (B) PMA-qPCR methods. Lines indicate the theoretical 1:1 line and the best fit line based on the experimental data. Conditions: Stationary phase *N. europaea*; 10 mM PBS, and pH 8.0.

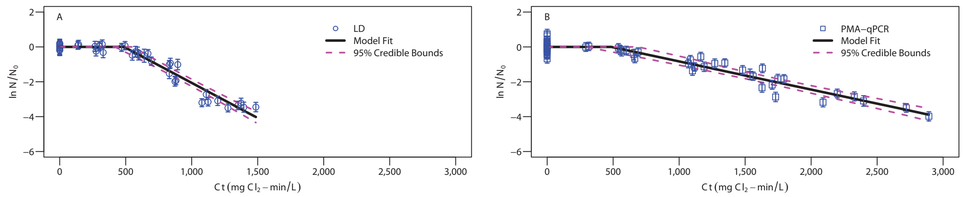

Figure 4. Delayed Chick-Watson model simulation and 95% credible bounds for (A) LD and (B) PMA-qPCR experimental data. Conditions: Stationary phase *N. europaea*; 10 mM PBS; pH 8.0; 25°C; and 5, 10, and 20 mg Cl_2/L monochloramine.

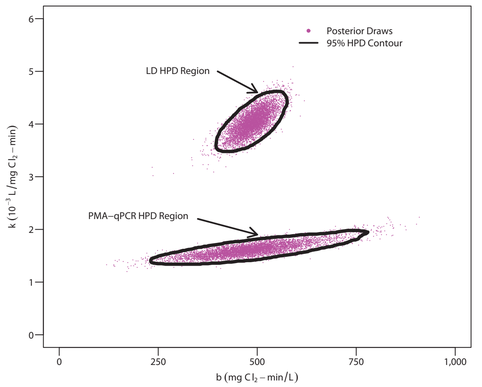

Figure 5. Kinetic parameter posterior distribution draws and associated 95% joint highest posterior density (HPD) region for the Delayed Chick-Watson model kinetic parameters estimated using the LD and PMA-qPCR experimental data for the PBS experiments.

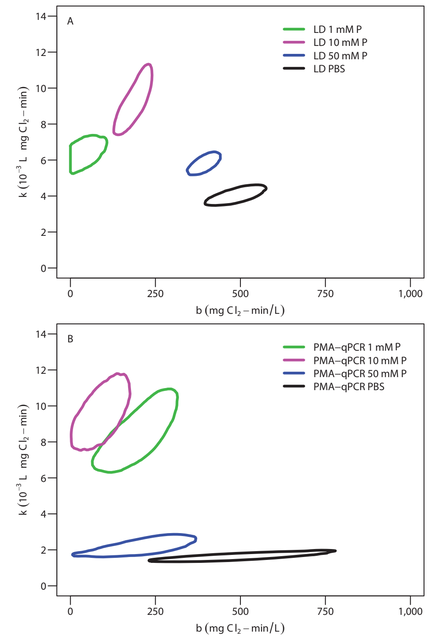

Figure 6. 95% joint highest posterior density region for the Delayed Chick-Watson model kinetic parameters estimated using (A) LD and (B) PMA-qPCR experimental data for the various buffer experiments (1 mM, 10 mM, and 50 mM Na_2PO_4 and 10 mM PBS).

Table 1. Disinfection kinetic parameter (b and k) summary

Experiment	Ionic Strength (mM)	LD (Value ± SD)		PMA-qPCR (Value ± SD)	
		b (mg-min/L)	*k* (10^{-3} L/mg-min)	*b* (mg-min/L)	*k* (10^{-3} L/mg-min)
1 mM Phosphate	2.8	37 ± 25	6.4 ± 0.42	200 ± 48	8.5 ± 0.91
10 mM Phosphate	27	190 ± 24	9.3 ± 0.80	91 ± 34	9.6 ± 0.84
50 mM Phosphate	120	394 ± 19	5.8 ± 0.25	230 ± 64	2.2 ± 0.25
10 mM PBS	150	490 ± 35	4.0 ± 0.23	490 ± 100	1.6 ± 0.12
Oldenburg et al.[1] 10 mM PBS	150	N/D	2.0	N/D	N/D

N/D – Not determined
[1]Adjusted to 25°C using activation energy for monochloramine of 77 kJ/mol

Conclusions

- Verified that LD and PMA-qPCR selectively measure viable cells in a mixture of *N. europaea* viable and nonviable cells
- Delayed Chick-Watson model simulated monochloramine disinfection kinetics
  - Initial lag phase represented by the lag coefficient (*b*)
  - Subsequent pseudo-first-order disinfection kinetics with a disinfectant rate constant (*k*)
- Disinfection kinetics experiments (PBS)
  - Similar lag coefficient (*b*) for both LD and PMC-qPCR
  - Significantly different disinfection rate constant (*k*) between LD and PMA-qPCR
  - PMA-qPCR based kinetics more conservative (i.e. slower disinfection) than LD based kinetics
- Buffer effect on disinfection kinetic experiments
  - Apparent competing effects between ionic strength and phosphate concentration
  - Disinfection kinetics increased (*b* decreased and *k* increased) in 10 mM phosphate versus 10 mM PBS
    - Greater effect on PMA-qPCR than LD based kinetic parameters
    - *k* → PMA-qPCR 6X & LD 2X
    - *b* → PMA-qPCR 0.2X & LD 0.4X
- Future applications of PMA-qPCR method
  - Experiments with mixed culture AOB representative of drinking water systems
  - Application to additional organisms and disinfectants

Acknowledgements

Andrew Carroll, Brandt Miller, John Regan, Mary Schoen, Mano Sivaganesan, Charlotte Smith, Gerald Speitel Jr., Mike Ware, Lauren Zalla, and the Microbial Contaminants Control Branch.

The U.S. Environmental Protection Agency, through its Office of Research and Development conducted the research described herein. Any opinions expressed are those of the authors and do not necessarily reflect the views of the Agency, therefore, no official endorsement should be inferred. Any mention of trade names or commercial products does not constitute endorsement or recommendation for use.