Background

Based on utility surveys, 30 to 63% of utilities practicing chloramination for secondary disinfection have nitrification afflictions (American Water Works Association 2006). Nitrification in drinking water distribution systems is undesirable and may result in water quality failures (e.g., disinfection by-products, coliform occurrences, or taste/odor formations) and subsequent noncompliance 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 microbially 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 (Noodt 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 (viable) 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 NaH2PO4, 130 mM NaCl); 25°C, and 5, 10, and 20 mg Cl2/L, monochloramine. Further experiments were conducted in additional phosphate buffers (1 mM, 10 mM, and 50 mM NaH2PO4) at pH 8.0, 25°C, and 5 mg Cl2/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.

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 WinLDOS (Bayesian analysis software) to estimate model parameters and their 95% credible bounds (Figure 4). Figure 5 displays the joint 95% highest posterior distribution draws and associated 95% posterior interval of LD parameter posterior distributions. The areas in Figure 5 highlight the greater uncertainty in the estimate of disinfection rate constant 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 estimates (Table 1) for both 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.

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 estimated 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 estimated (PBS)
  - Similar lag coefficient (*b*) for both LD and PMA-qPCR
  - Significantly different disinfection rate constant (*k*) between LD and PMA-qPCR
- PMA-qPCR based kinetic parameters more conservative (i.e. slower disinfection) than LD based kinetic parameters
- Buffer effect on disinfection kinetics experiments
  - Approximate computing efficiency loss with increasing 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

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