Monochloramine Cometabolism by Ammonia-Oxidizing Bacteria [Project #4341]

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PRINCIPAL INVESTIGATORS:
Gerald E. Speitel Jr., Juan P. Maestre, and David G. Wahman

OBJECTIVES

The hypothesis of this research was that under some conditions, monochloramine cometabolism causes disinfectant loss in distribution systems, and is a defense mechanism that allows nitrifiers to persist in pipe-wall biofilm. The research objectives were to (1) define the relative significance of monochloramine cometabolism in causing disinfectant loss over the typical range of conditions encountered in drinking water distribution systems, and (2) provide a mathematical description of monochloramine cometabolism for inclusion in distribution system water quality models (e.g., EPANET-MSX).

BACKGROUND

Chloramine use is widespread in U.S. drinking water distribution systems as a secondary disinfectant. In a recent survey of water utilities, 30% of respondents used chloramines to maintain distribution system residual (AWWA Water Quality and Technology Division Disinfection Systems Committee 2008), while other recent surveys suggest that between 8% and 12% of drinking water utilities are contemplating a switch to chloramination (Seidel et al. 2005, AWWA Water Quality and Technology Division Disinfection Systems Committee 2008). Upon implementation of the Stage 2 Disinfectants and Disinfection By-products Rule, chloramination for secondary disinfection in the United States is predicted to increase to 57% of all surface and 7% of all ground water treatment systems (EPA 2005). While beneficial for controlling regulated disinfectant by-product formation, chloramination may promote the growth of nitrifying bacteria (i.e., ammonia-oxidizing bacteria [AOB] and nitrite-oxidizing bacteria [NOB]). This occurs because of naturally occurring ammonia, residual ammonia remaining from initial chloramine formation, ammonia released from chloramine decay, oxidation of natural organic matter (NOM), corrosion, pipe surface reactions, and nitrite (NO₂⁻) oxidation under various conditions in chloraminated water systems (Wilczak et al. 1996, AWWA 2013). Nitrification (i.e., microbially-mediated ammonia and nitrite oxidation) is a significant problem in many distribution systems where chloramines are used as the secondary disinfectant. For example, Wilczak et al. (1996) surveyed 67 medium and large utilities practicing chloramination and found that 63% of them experienced nitrification to some degree, and about 25% had moderate to severe nitrification.
problems. Thus, nitrification control is a major issue in practice and likely will become an issue of growing importance as the prevalence of chloramination increases.

A variety of factors may influence the likelihood of nitrification episodes, including disinfectant concentration, chlorine to nitrogen (Cl\textsubscript{2}:N) mass ratio, free ammonia concentration, temperature, and detention time in distribution systems. In addition to ammonia metabolism, AOB also can cometabolize a wide variety of chemicals via the non-specific enzyme, ammonia monooxygenase (AMO). Cometabolism is defined as the fortuitous biodegradation of a target chemical through reactions catalyzed by non-specific microbial enzymes. For example, Hooper et al. (1997) provided a summary detailing that the AMO enzyme of \textit{Nitrosomonas europaea} can cometabolize over 35 halogenated chemicals, using ammonia as the growth substrate. Recent research has shown that \textit{N. europaea}, as well as mixed cultures of nitrifiers present in natural waters, treatment plants, and distribution systems, can cometabolize the four regulated trihalomethanes (THMs) at rates that are relevant in drinking water treatment applications (Wahman et al. 2005, Wahman et al. 2006a). Based on its structural similarity to ammonia and other chemicals that AOB can cometabolize, it is highly likely that monochloramine is similarly cometabolized. As proposed by Woolschlager (2000), AMO would convert monochloramine to chlorohydroxylamine, which would be converted by hydroxylamine oxidoreductase (HAO) to nitrite and chloride in an analogous fashion to ammonia metabolism. Abiotically, chlorohydroxylamine may also rapidly disassociate into nitroxyl (HNO) and chloride, with HNO subsequently reacting by a series of competing reactions, resulting in a mixture of nitrate, nitrite, nitrous oxide, and nitrogen gas (Giles 1999, Wahman et al. 2014). To date, the role that monochloramine cometabolism plays in determining monochloramine residual concentrations in drinking water distribution systems subject to nitrification has not been considered in practice.

**APPROACH**

The experimental plan consisted of two phases. The first phase focused on batch kinetic experiments to measure monochloramine cometabolism kinetics, characterize enzyme competition with ammonia, and evaluate the environmental condition impact on cometabolism kinetics. The major goals of Phase 1 were:

1. Demonstrate conclusively that AOB can cometabolize monochloramine;
2. Measure monochloramine cometabolism kinetics;
3. Determine if enzyme competition between monochloramine and ammonia is important, and, if so, quantitatively characterize the competition;
4. Determine if monochloramine cometabolism relies on the presence of ammonia to provide the reducing power for driving cometabolism;
5. Identify the appropriate kinetic model for describing monochloramine cometabolism; and
6. Estimate the values of the rate constants in the monochloramine cometabolism model.

The results of Phase 1 were extended to conditions typical of water distribution systems in Phase 2 through experiments with annular bioreactor systems designed to simulate distribution system hydraulics and continuous-flow conditions. The major goals of Phase 2 were:

1. Document monochloramine cometabolism occurrence under distribution system nitrification conditions;
2. Characterize the relative significance of monochloramine cometabolism in distribution system nitrification episodes as a function of environmental conditions (e.g., pH, Cl₂:N mass ratio, monochloramine concentration); and

3. Incorporate nitrification and monochloramine kinetic expressions into a model that accounts for distribution system hydraulics and the possibility of both biofilm and suspended growth of nitrifying bacteria.

RESULTS/CONCLUSIONS

The Phase 1 experiments demonstrated that monochloramine cometabolism by *N. europaea* occurs at significant rates under conditions relevant to drinking water systems. Notably, the monochloramine cometabolism rate was comparable to the ammonia metabolism rate. As a result, monochloramine cometabolism was an important contributor to monochloramine loss, accounting for 30% to 60% of the loss. By extension, other AOB should be able to cometabolize monochloramine, and monochloramine cometabolism is likely to be an important contributor to the accelerated monochloramine loss observed at the onset of nitrification in drinking water distribution systems. Monochloramine cometabolism was simulated by a pseudo-first-order reductant model, providing a basis for including monochloramine cometabolism in distribution system water quality models. In this model, the monochloramine cometabolism rate increases with increasing free ammonia concentration, providing an additional motivation to limit the free ammonia concentration in drinking water distribution systems.

The Phase 1 experiments carried out with a mixed culture microbial community demonstrated that monochloramine cometabolism occurs in mixed cultures under conditions relevant to drinking water systems. This research also showed that monochloramine cometabolism is a relevant process leading to monochloramine loss, accounting for 30% of the monochloramine loss in these experiments. Monochloramine reactions with biomass accounted for 60% of the loss, and reactions with utilization-associated products (UAPs) generally accounted for approximately 10% of the monochloramine loss. UAPs are simple organics produced as AOB oxidize ammonia to nitrite and are available to react with monochloramine. Because of the small contribution of the UAP reactions to overall monochloramine loss, the estimate of the UAP rate constant (k_uap) had considerable uncertainty associated with it, and the 95% confidence interval passed through zero, further indicating the small contribution of the UAP reactions. The contributions of autodecomposition and nitrite reactions were negligible, as designed by virtue of the mid-range pH and low Cl₂:N ratios. Consequently, monochloramine cometabolism is likely to be an important contributor to the accelerated monochloramine loss observed at the onset of nitrification in drinking water distribution systems.

In Phase 2A, nitrifying biofilms were grown in the absence of monochloramine. For these growth conditions, four subsequent batch cometabolism experiments showed a shutdown of nitrification and monochloramine cometabolism at the two higher initial monochloramine concentrations of 1.0 and 1.6 mg Cl₂/L. In Phase 2B, nitrifying biofilms were grown in the presence of a low monochloramine concentration. For these growth conditions, three subsequent batch cometabolism experiments demonstrated steady nitrification throughout the experiments for initial monochloramine concentrations ranging from 0.62 to 1.1 mg Cl₂/L. These differing results are suggestive that nitrifying biofilm developed in the presence of monochloramine may be more tolerant of increasing monochloramine concentrations than those that develop in the absence of monochloramine.

The two cometabolism experiments in Phase 2A, for which nitrification ceased, were excluded from the subsequent modeling effort. For the five remaining experiments, the model fit
both the ammonia and monochloramine concentration profiles well in the cometabolism experiments. The most important output from the modeling effort was an estimate of the monochloramine cometabolism rate constant. Estimation of the rate constant was possible in three of the experiments, while in the other two experiments, the estimate of the rate constant was not statistically different from zero. The estimated rate constants agreed very well with each other and with the value determined in experiments with the mixed culture suspensions. Thus, the annular reactor experiments provide further evidence that monochloramine cometabolism may be a significant monochloramine loss mechanism under conditions encountered in drinking water distribution systems.

While the model fit the two Phase 2B experiments that had higher initial monochloramine concentrations (0.81 and 1.14 mg Cl₂/L, respectively), abiotic reactions dominated under these conditions, preventing an accurate estimate of the monochloramine cometabolism rate constant. Monochloramine’s reaction with biomass dominated as the pathway for monochloramine loss in these two experiments. In contrast, 25 to 40% of the monochloramine loss was attributable to the cometabolism pathway in the other experiments. In all experiments, the reaction pathway with UAPs was a notable contributor to monochloramine loss (20 to 30%).

Overall, this research highlights a threshold effect for monochloramine residual concentration. At sufficiently high concentrations (~1 mg Cl₂/L in this research), nitrification will be well controlled, and monochloramine cometabolism will be a negligible contributor to monochloramine loss. When the monochloramine concentration is insufficient, nitrification will commence, and monochloramine cometabolism may be a significant reaction pathway for monochloramine loss. These findings reinforce recommendations for the maintenance of minimum monochloramine residuals in drinking water distribution systems. The Surface Water Treatment Rule (EPA 1989) only requires the maintenance of a “detectable” residual, which clearly is inadequate for nitrification control. Fortunately, some states (e.g., Texas) specify minimum residual concentrations that must be maintained.

APPLICATIONS/RECOMMENDATIONS

A variety of factors may influence the likelihood of nitrification episodes in chloraminated drinking water distribution systems, including disinfectant concentration, Cl₂:N mass ratio, total free ammonia concentration, temperature, and detention time in the distribution system. This report demonstrated a yet uncharacterized mechanism of monochloramine loss through monochloramine cometabolism, using suspended pure (N. europaea) and mixed culture AOB and biofilm associated AOB grown both with and without monochloramine presence. Furthermore, the estimated cometabolism kinetic parameters suggest that cometabolism kinetics are fast enough to contribute to rapid monochloramine loss in actual drinking water distribution systems during nitrification episodes. This report has several implications for utilities using or planning to use chloramines for secondary disinfection with regards to controlling nitrification.

First, it was evident from the kinetic experiments that there were monochloramine concentrations above which rapid negative impacts to ammonia oxidation were seen that could not be explained by assuming first-order (i.e., Chick-Watson) inactivation kinetics. Based on the experiments conducted, a monochloramine concentration above approximately 1.0 mg Cl₂/L resulted in significant and immediate impacts to ammonia oxidation. This concentration corresponds to what is typically recommended for minimum monochloramine residual maintenance in distribution systems (AWWA 2013), which typically exceeds regulatory requirements for minimum required disinfectant residuals (i.e., “detectable” in a majority of states). Therefore, when trying to control nitrification, it is important for utilities to realize that
simply meeting regulations may not be sufficient to meet water quality requirements based on nitrification control. It should be further noted that even though these AOB were inactive using ammonia oxidation as the inactivation measure, they were still viable using cell membrane integrity as the inactivation measure. This implies that if the residual would decrease, these same AOB may recover. Additionally, biofilm associated AOB were found to be more resistant to monochloramine exposure (based on ammonia oxidation) than the suspended pure culture or mixed culture.

Second, at least for the pure culture *N. europaea*, a reductant model described the monochloramine cometabolism kinetics. This implies that if free ammonia is maintained at a low enough level, then monochloramine cometabolism will not occur. This provides yet another reason to try and minimize the free ammonia concentration in distribution systems, limiting this monochloramine loss pathway. The minimum acceptable free ammonia concentration will be system specific, involving a balancing between the risk of nitrification and other water quality goals (e.g., monochloramine stability).

Third, chloramine demand pathways associated with biotic related mechanisms (e.g., cometabolism, biomass reaction, and UAP reaction) were much greater than abiotic chloramine loss mechanisms for the water quality used (pH 8.3, 4 mM carbonate buffer). This further emphasizes that utilities should manage distribution system operation to minimize biofilm development (e.g., biofilm on pipe walls or storage tank sediments) as these are likely causes of disinfectant residual loss that become exacerbated as the bacteria become more active.

Fourth, the biological process matrices presented in this report and associated kinetic parameter estimates provide the necessary information for incorporation of monochloramine cometabolism into water quality models (e.g., EPANET-MSX) to improve simulations used to investigate possible operational condition impacts on nitrification occurrence.

In terms of interpreting the results for actual distribution systems, the biofilms in these experiments were grown on polycarbonate slides (i.e., non-reactive), which would best represent growth on plastic pipes. In actual distribution systems where metal (e.g., copper, iron, lead) pipe materials are common, the monochloramine concentrations required to cause similar impacts to ammonia oxidation as seen in this research are expected to be greater because of the reactivity of the metals. Therefore, monochloramine residual guidance provided by these experiments should be viewed as a minimum level. Typically, recommendations for controlling nitrification focus on maintaining monochloramine residual and minimizing free ammonia. As shown in this report, these recommendations would also seek to minimize monochloramine cometabolism by AOB.