WATER RESEARCH 68 (2015) 719–730

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Hydroxylamine addition impact to *Nitrosomonas europaea* activity in the presence of monochloramine

David G. Wahman [a,*], *Gerald E. Speitel Jr.* [b]

[a] *United States Environmental Protection Agency, Office of Research and Development, Cincinnati, OH 45268, USA*
[b] *University of Texas at Austin, Department of Civil, Architectural and Environmental Engineering, Austin, TX 78712, USA*

ARTICLE INFO

Article history:
Received 5 May 2014
Received in revised form
24 September 2014
Accepted 24 October 2014
Available online 1 November 2014

Keywords:
Hydroxylamine
Monochloramine
Drinking water
Nitrification
Nitrosomonas europaea

ABSTRACT

In drinking water, monochloramine may promote ammonia–oxidizing bacteria (AOB) growth because of concurrent ammonia presence. AOB use (i) ammonia monooxygenase for biological ammonia oxidation to hydroxylamine and (ii) hydroxylamine oxidoreductase for biological hydroxylamine oxidation to nitrite. In addition, monochloramine and hydroxylamine abiotically react, providing AOB a potential benefit by removing the disinfectant (monochloramine) and releasing growth substrate (ammonia). Alternatively and because biological hydroxylamine oxidation supplies the electrons (reductant) required for biological ammonia oxidation, the monochloramine/hydroxylamine abiotic reaction represents a possible inactivation mechanism by consuming hydroxylamine and inhibiting reductant generation. To investigate the abiotic monochloramine and hydroxylamine reaction's impact on AOB activity, the current study used batch experiments with *Nitrosomonas europaea* (AOB pure culture), ammonia, monochloramine, and hydroxylamine addition. To decipher whether hydroxylamine addition benefitted *N. europaea* activity by (i) removing monochloramine and releasing free ammonia or (ii) providing an additional effect (possibly the aforementioned reductant source), a previously developed cometabolism model was coupled with an abiotic monochloramine and hydroxylamine model for data interpretation. *N. europaea* maintained ammonia oxidizing activity when hydroxylamine was added before complete ammonia oxidation cessation. The impact could not be accounted for by monochloramine removal and free ammonia release alone and was concentration dependent for both monochloramine and hydroxylamine. In addition, a preferential negative impact occurred for ammonia versus hydroxylamine oxidation. These results suggest an additional benefit of exogenous hydroxylamine addition beyond monochloramine removal and free ammonia release, possibly providing reductant generation.

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* *Corresponding author*. USEPA, 26 W. Martin Luther King Dr., Cincinnati, OH 45268, USA. Tel.: +1 513 569 7733; fax: +1 513 487 2543.
E-mail address: wahman.david@epa.gov (D.G. Wahman).
http://dx.doi.org/10.1016/j.watres.2014.10.054
0043-1354/Published by Elsevier Ltd.

1. Introduction

The implementation of the Stage 2 Disinfectants and Disinfection Byproducts Rule (i.e., ~2015) is expected to increase monochloramine (NH_2Cl) use for secondary disinfection in the United States to 57% of all surface and 7% of all ground water treatment systems (USEPA, 2005). Monochloramine use may promote ammonia–oxidizing bacteria (AOB) growth because of residual ammonia remaining from initial monochloramine formation and ammonia released from monochloramine decay and demand reactions (American Water Works Association, 2013). Monochloramine residual loss is often associated with nitrification onset and may result in noncompliance with existing regulations (e.g., Surface Water Treatment Rule); therefore, understanding nitrification and its control in drinking water distribution systems is of practical importance (American Water Works Association, 2013).

Nitrosomonas europaea is the most studied AOB pure culture. Fig. 1 depicts *N. europaea*'s ammonia oxidation pathway (Sayavedra-Soto and Arp, 2011) along with possible impacts from monochloramine exposure. For ammonia (NH_3) oxidation, *N. europaea* uses two enzymes in two reaction steps to form nitrite (NO_2^-) eventually: (i) the membrane–bound ammonia monooxygenase (AMO) enzyme catalyzes ammonia oxidation to hydroxylamine (NH_2OH) and (ii) the periplasmic–residing hydroxylamine oxidoreductase (HAO) enzyme catalyzes hydroxylamine oxidation to nitrite. For AMO, the reductant (electron [e^-]) source to sustain ammonia oxidation results from hydroxylamine oxidation (Fig. 1). Two of the four electrons resulting from hydroxylamine oxidation are cycled back to AMO for ammonia oxidation, while the other two electrons are used for other cellular processes.

When monochloramine is present with *N. europaea*, at least four possible monochloramine loss pathways exist (Fig. 1): (Pathway A) reaction with various cellular components, leading to *N. europaea* inactivation (Holder et al., 2013; Jacangelo and Olivier, 1985; Jacangelo et al., 1991, 1987a, 1987b; Wahman et al., 2009); (Pathway B) biological transformation by *N. europaea* (Maestre et al., 2013) through cometabolism (the fortuitous biodegradation of a target chemical [i.e., monochloramine] through reactions catalyzed by nonspecific microbial enzymes [i.e., AMO]); (Pathway C) abiotic reaction with nitrite (Johnson and Margerum, 1991; Margerum et al., 1994; Wahman and Speitel, 2012); and (Pathway D) abiotic reaction with hydroxylamine (Aoki et al., 1989; Ferriol et al., 1986; Giles, 1999; Robinson et al., 2005; Wahman et al., 2014). The four monochloramine loss pathways provide *N. europaea* with both possible benefits and detriments. All pathways may benefit *N. europaea* by reducing monochloramine (i.e., the disinfectant) and, except for cometabolism, releasing free ammonia as *N. europaea*'s growth substrate (Jacangelo et al., 1987a, 1987b; Maestre et al., 2013; Vikesland et al., 1998; Wahman and Speitel, 2012; Wahman et al., 2014). Even though all pathways may benefit *N. europaea* by reducing monochloramine, two pathways may have competing negative impacts to *N. europaea* (Pathways A and D, Fig. 1). First, monochloramine's direct reaction with cellular components (Pathway A, Fig. 1) would lead to inactivation after enough cell damage has occurred. Second, because biological hydroxylamine oxidation supplies the electrons required for biological free ammonia oxidation,

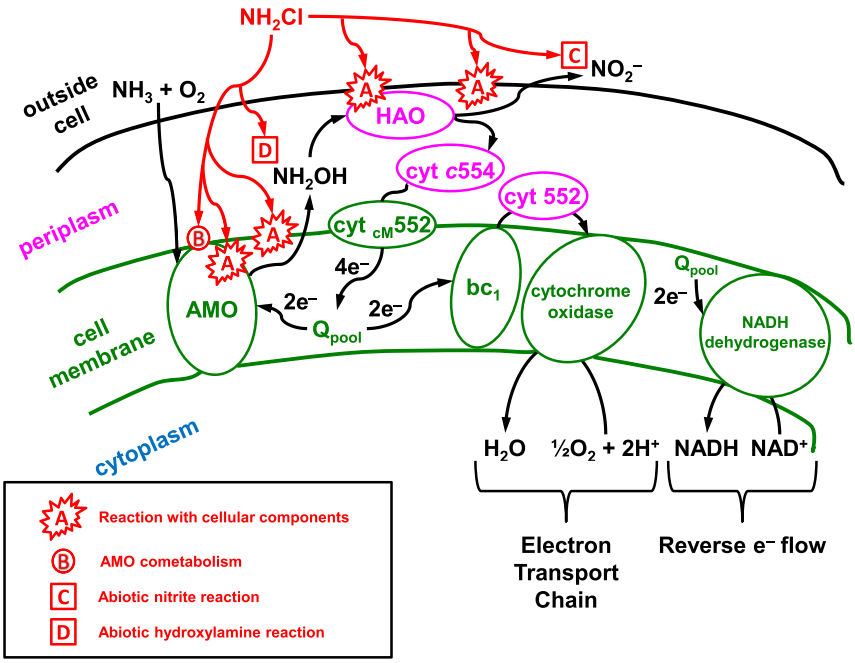

Fig. 1 – *N. europaea* oxidation model adapted from Sayavedra-Soto and Arp (2011). Possible impacts of monochloramine (NH_2Cl) are also displayed (Pathways A, B, C, and D). All monochloramine pathways consume monochloramine and, except AMO cometabolism, release ammonia (NH_3). Nitrogen containing end products for each pathway: A (ammonia), B (nitrite), C (ammonia and nitrate), and D (ammonia, nitrite, nitrate, nitrous oxide, and nitrogen gas). AMO, ammonia monooxygenase; bc_1, complex III; cyt, cytochrome; HAO, hydroxylamine oxidoreductase; Q_{pool}, ubiquinone pool.