Kinetic Analysis Implicates Nitrous Oxide as a Potent Inhibitor of the Bacterial Reductive Dehalogenation Process

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Background/Objectives. Intense usage of nitrogen (N) fertilizer has resulted in increased nitrate concentrations in groundwater. Under anoxic conditions, nitrate is reduced via microbial denitrification to nitrous oxide (N_2O) and dinitrogen (N_2). As a consequence, groundwater N_2O concentrations exceed the equilibrium concentration with atmospheric N_2O (~7 nM at 20 °C) by up to 4 orders of magnitude. N_2O reacts with certain metalloorganic prosthetic groups such as cobalt- (Co-) containing corrinoids (e.g., vitamin B_{12}). The chemical reaction of N_2O with Co(I) can cause damage to corrinoid-dependent enzyme systems and thus impact an organism's metabolism. Organohalide-respiring bacteria require corrinoid for assembling functional reductive dehalogenases, which are the key enzyme system catalyzing reductive dechlorination and detoxification of priority pollutants. For example, *Geobacter lovleyi* strain SZ dechlorinates tetrachloroethene (PCE) to *cis*-1,2-dichloroethene (*c*DCE), and *Dehalococcoides mccartyi* strain BAV1 dechlorinates *c*DCE via vinyl chloride (VC) to environmentally benign ethene. As global fertilizer usage increases, the impact of elevated N2O concentrations on relevant microbial processes in (contaminated) aquifers must be understood.

Approach/Activities. The effect of N_2O ranging from 5 to 100 µM on the corrinoid-dependent reductive dechlorination activities in *Geobacter lovleyi* strain SZ and *Dehalococcoides mccartyi* strain BAV1 were examined in whole cell suspension assays. A Michaelis-Menten single substrate model was used to illustrate the inhibitory effects of N_2O on the reductive dechlorination process.

Results/Lessons Learned. Low concentrations (9.5 µM) of N_2O impacted PCE and cDCE reductive dechlorination rates and extents in strain SZ and in strain BVA1 cultures, respectively, suggesting that N_2O is a potent inhibitor of corrinoid-dependent reductive dechlorination. The most pronounced inhibition was observed for the VC reductive dechlorination step in strain BAV1 cultures, and only negligible amounts of VC were dechlorinated at N_2O concentrations exceeding 19 µM. Following removal of 95 µM N_2O from inhibited cultures, the reductive dechlorination activity was fully restored in strain SZ cultures. In contrast, recovery of VC dechlorination activity was not observed in strain BAV1 cultures even after extended incubation periods when the initial N_2O concentration exceeded 29 µM. The kinetic parameters for both isolates in the presence of 0-60 µM N_2O were determined using the Michaelis-Menten model. In strain SZ assays, the whole-cell half-saturation constant (K_s) of 25.1±2.9 µM for PCE was not affected by 60 µM N_2O, but ~26 µM N_2O reduced the maximum PCE dechlorination rate V_{max} of 76.3±2.6 nmol Cl- released/min/mg protein by 50%. A measured inhibitory constant (K_i) of 26.0± 4.9 µM indicated that PCE dechlorination rate (i.e., *Vmax*) is impacted at environmentally relevant N_2O concentrations. Similar results were observed for *c*DCE dechlorination in strain BAV1 assays. In the absence of N_2O, a Ks value of 23.1±4.1 µM and a *Vmax* value of 121.3±6.2 nmol Cl- released/min/mg protein were determined. When exposed to 17.7-24.7 µM N_2O, the *Vmax* of *c*DCE dechlorination decreased ~50%, and a K_i value of 21.2±3.5 µM was determined. These kinetic measurements highlight that N_2O inhibition of reductive dechlorination must be expected at environmentally relevant concentrations, particularly in aquifers receiving agricultural nitrate runoff. Considering the commonality of elevated nitrate concentrations in many aquifers, the findings provide an explanation for stalled or incomplete reductive dechlorination at sites impacted with chlorinated pollutants. Thus, groundwater monitoring regimes should include nitrate and N_2O measurements, so that potential inhibition and VC stalls can be explained and predicted.