The Effect of pH on Nitrification by *Nitrosomonas europaea*

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When a distribution system is subjected to chloramination, there is the potential for nitrifying bacteria such as *Nitrosomonas* species to use the available ammonia for chemolithic growth, i.e. bacteria use the ammonia as an energy source by converting it to nitrite. This contrasts with heterotrophic bacterial use of organic compounds.

The bacteria may colonize and multiply on the interior surface of distribution pipes and other components to create biofilms. These biofilms consequently may produce taste and odor problems in the water. They also may continuously react with the water's residual disinfectant, decreasing it in the distal parts of the system.

To eliminate biofilms of nitrifying bacteria, it is necessary to annually convert the distribution system to chlorination. This eliminates the ammonia food source for the bacteria and the chlorine more effectively kills the organisms.

Des Moines Water Works is a lime softening utility. It provides water to its distribution system with a pH between 9.2 and 9.7. Because some bacteria are inhibited at higher pHs a study was designed to determine what effect, if any, distribution water pH would have on nitrification and growth by *Nitrosomonas europaea*. This information has special value for DMWW, since it uses chloramination for disinfection in its Southeast Polk Rural Water system.

Method:

An American Type Culture Collection strain of *Nitrosomonas europaea* (ATCC® 25978) was purchased for this experiment. A growth medium was made using filter effluent water of the DMWW Fleur treatment plant with added ammonium sulfate. The ammonia concentration was adjusted to 0.5mg/liter (expressed as NH_3). This was closely analogous to the facility's chloraminated distribution water, minus the undesirable interfering variable of chlorine.

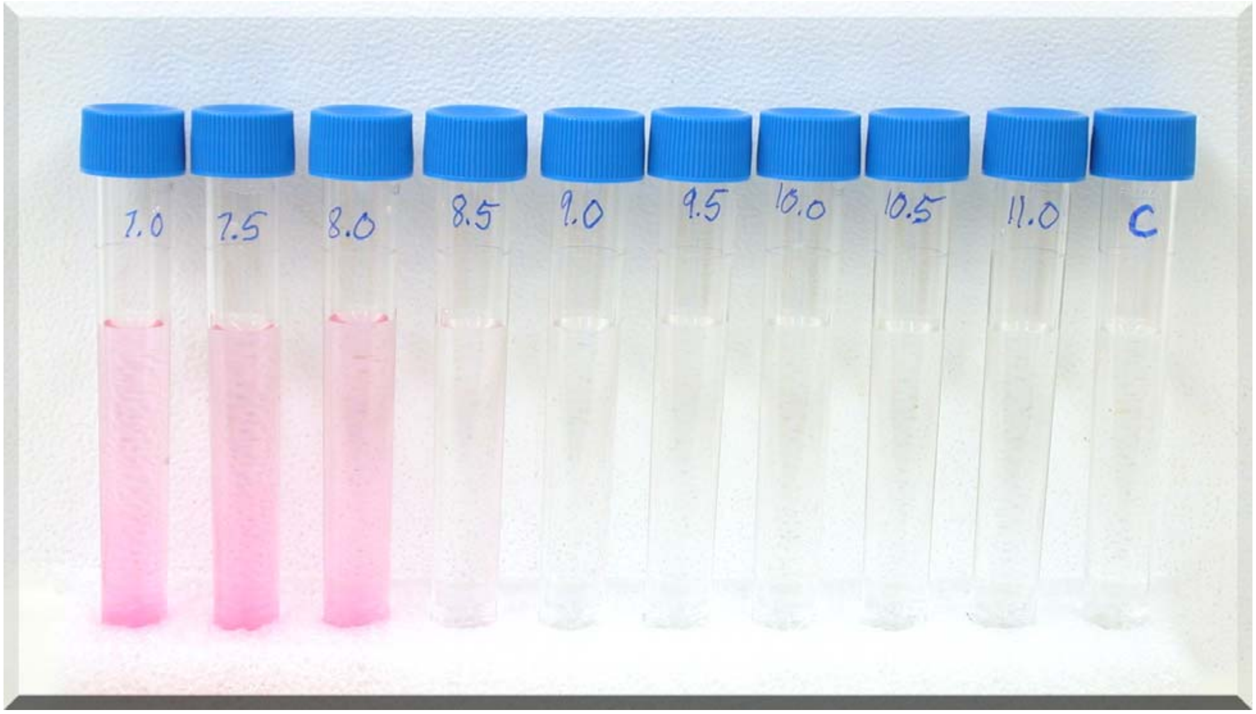
Five milliliters of the growth medium were added to each of nine test tubes and nine control tubes. The pHs of each set of tubes were 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, and 11.0.

Each test tube was inoculated with *Nitrosomonas europaea*. The control tubes were not inoculated with bacteria. The sets were then incubated for three days at 23°C (room temperature).

Three drops of 0.8% sulfanilic acid, and three drops of N,N-dimethyl-alpha-naphthylamine were added to the medium of each test and control tube. Each tube was checked to confirm the pH was lowered sufficiently with the sulfanilic acid to allow the color reaction to occur. All tubes were confirmed to be at a pH of 3.4±0.2. The tubes were then held for three hours for complete reaction of the reagent.

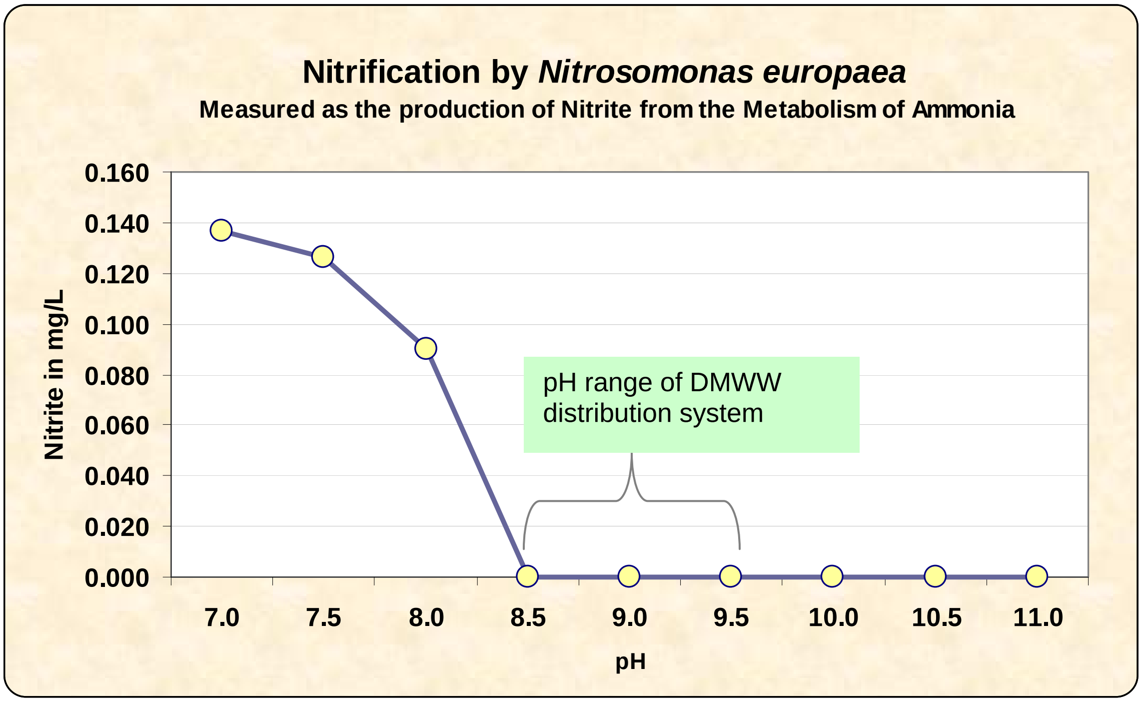
Development of a pink color confirms the presence of nitrite as a metabolic end-product of ammonia.

Results:
The most intense pink color developed in the tubes that had an initial pH of 7.0, 7.5 and 8.0. The medium at pH 8.5 had much less pink color and the other tubes looked colorless to the eye. All of the control media looked colorless.



To better quantify the nitrite present, color was measured spectrophotometrically. When compared to a nitrite standard using the same medium, the results were as follows:

Test Medium pH	7.0	7.5	8.0	8.5	9.0	9.5	10.0	10.5	11.0
Absorbance at 530nm	0.206	0.191	0.139	0.007	0.000	0.000	0.000	0.013	0.006
Nitrite present mg/L	0.137	0.126	0.090	0.000	0.000	0.000	0.000	0.000	0.000



Summary:

The test media with pH values of 8.5 and above effectively inhibited nitrification by *Nitrosomonas europaea*. It is likely that the growth of *N. europaea* was inhibited too.

When DMWW treats water with lime softening the pH ranges between 10.0 and 11.0. Later in treatment, carbon dioxide is added to lower the pH to 9.6. The pH continues to decrease to approximately 9.2 as the water progresses through the clear well and into the distribution system. Once in the distribution system for several days, the pH can further drop to 8.8 or lower, but it still maintains a relatively high value when compared to the distribution system water of other utilities.

By supplying the distribution system with water at a relatively high pH, it is reasonable to extrapolate from this study that nitrifying bacteria will likely be inhibited from growing. This makes it likely that an annual switch to chlorine will not be necessary.

If, indeed, the need to switch to chlorine in order to eliminate nitrifying bacteria does not exist, much labor by DMWW employees will be saved. This year's switch from chloramination treatment to chlorine treatment in the Southeast Polk Rural Water system required that the control center operators drain the system's water towers of chloraminated water and refill them with chlorinated water. In addition, two distribution employees spent several days flushing numerous branches of the system. The flushing was performed to ensure that each line was fully chlorinated. These procedures may no longer be necessary in future years.

Omission of an annual chlorination procedure will also keep the consistency of water conditions for the customers better maintained.

To definitively determine if annual shocking of the system with free chlorine is necessary, cultures for nitrifying bacteria will be performed on the distribution water.