

High pH Inhibits Nitrifying Bacteria

Des Moines Water Works used periodic testing and analysis of its system's nitrifying bacteria to determine if conversion to free chlorine was warranted.

BY DENNIS R. HILL AND TERRY WEBSTER

FREE AMMONIA IN chloraminated water distribution systems promotes growth of nitrifying bacteria, which can generate nitrite, deplete disinfectant, and cause taste and odor problems. Nitrifying bacteria metabolize ammonia into nitrite and are classified as chemolithotrophic (chemical and stone eaters). They derive nutrients from inorganic compounds and don't require an external organic food source, unlike more common heterotrophic bacteria, which subsist on organic food sources.

Significant growth of nitrifying bacteria may be inhibited by the high finished water pH that results from lime softening, enabling utilities to avoid annual conversion to free chlorine to eliminate biofilms composed of these organisms. An elevated nitrite level in a utility's water distribution system indicates substantial nitrifying activity, requiring conversion to free chlorine long enough to eliminate the bacteria's potential biofilms. This usually requires flushing dead-end and low-use mains to effectively chlorinate those sites. This expensive and labor-intensive process may require a month or more to accomplish.

ASSESSING FREE CHLORINE CONVERSION

Des Moines (Iowa) Water Works operates a chloraminated rural water distribution

system. The system converted to free chlorine during winter because of concerns about biofilm formation by nitrifying bacteria, despite low levels of nitrite in the water. To assess whether temporary free chlorine conversion was necessary, the DMWW microbiologist designed and implemented three procedures that enriched the organisms in an ammonia-containing medium. The detection of nitrite as an end product in this medium was proof of growth and nitrification.

The first procedure involved determining the pH sensitivity of an American Type Culture Collection strain of *Nitrosomonas europaea* (ATCC 25978). The second determined the presence of nitrifying bacteria in the rural distribution system of interest. The third procedure assessed possible biofilms at one system site.

The rural system's source entry point is about 15 mi from the treatment plant. During periods of chloramination, chlorine levels are boosted to 2.0 mg/L at the source entry point, and 0.5 mg/L of ammonia is fed as ammonium sulfate. The distribution system is composed of PVC piping less than 20 yr old.

SENSITIVITY STUDY

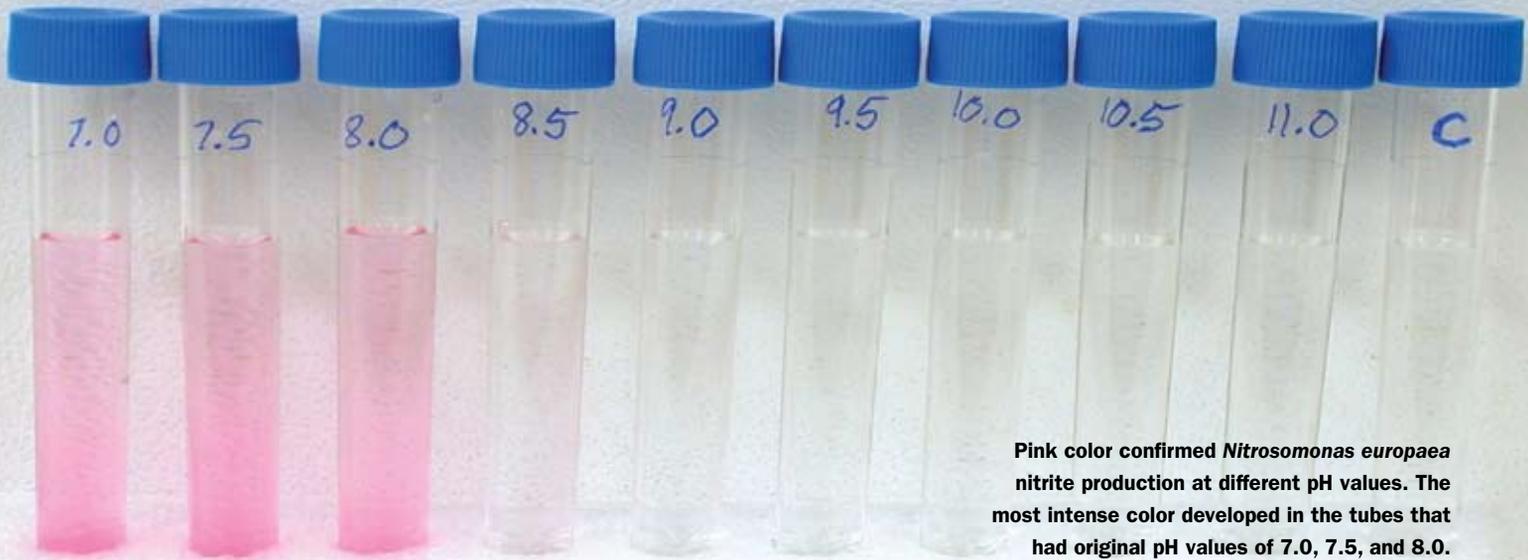
To determine the effect of pH on an ATCC strain of *N. europaea*, a growth medium was created by adding ammonium sulfate to filter effluent water that had been

lime softened to a hardness of 150 mg/L. Ammonia nitrogen ($\text{NH}_3\text{-N}$) concentration in the medium was 0.5 mg/L. Each of nine test tubes and nine control tubes had 5 mL of growth medium added to it. The pH of each tube was adjusted to produce 0.50 intervals from 7.0 to 11.0.

Each test tube, except for the control tubes, was inoculated with *N. europaea*. The sets were then incubated at 23°C (room temperature) for three days, the approximate water turnover time in the mains at any one point and the time required for the media-adapted stock organism to convert ammonia to nitrite in pronounced amounts in pH neutral conditions.

Three drops of N, N-dimethyl-alpha-naphthylamine and three drops of 0.8 percent sulfanilic acid were added to each test and control tube. Each tube was checked to confirm that the pH was lowered sufficiently (to 3.4 ± 0.2) with the sulfanilic acid to allow a confirmatory color reaction to occur. The tubes were then held for 3 hr to observe the complete reagent reaction. Development of a pink color confirmed the presence of nitrite as a metabolic end-product of ammonia.

The most intense pink color developed in the tubes that had original pH values of 7.0, 7.5, and 8.0. The 8.5 pH medium was slightly pink, and the remaining tubes



Pink color confirmed *Nitrosomonas europaea* nitrite production at different pH values. The most intense color developed in the tubes that had original pH values of 7.0, 7.5, and 8.0.

looked colorless to the eye. All of the control media looked colorless.

To better quantify the amount of nitrite present, color was measured spectrophotometrically at 530 nm, the appropriate wavelength for quantifying maximum absorbency for pink. The nitrite generated by growth was 0.137 mg/L at pH 7.0, 0.126 mg/L at pH 7.5, 0.090 mg/L at pH 8.0, and 0.000 mg/L at higher pH levels.

The test media with pH greater than 8.5 inhibited nitrification by *N. europaea* and likely inhibited the strain's growth as well. Lime softening treatment at the DMWW treatment plant is conducted at pH 10.0 to 11.0, depending on time of year and source water quality. Following softening, recarbonation with carbon dioxide lowers the pH to about 9.5.

NITRIFYING BACTERIA STUDY

To determine if nitrifying bacteria existed in the chloraminated system, 100-mL samples were collected from faucets distributed throughout the system and identified as sites 1-5. A minimal amount of sodium thio-sulfate was added to remove any residual chlorine that could inhibit bacteria growth. The pH was adjusted to 7 to allow pH-sensitive nitrifying bacteria, if present, to grow. Nitrite analysis was conducted every three days throughout the incubation periods to ensure detection of nitrite generated from metabolized ammonia, before being further metabolized to nitrate or nitrogen by other bacteria. At times, samples developed nitrite after a few days, only to test negative for it later in the incubation period. Ammonium sulfate was added to maintain ammonia concentrations at 0.5 mg/L to lengthen the experiment's duration.

A positive control was made from a composite of each sample. Ammonium sulfate and *N. europaea* were added to the control. Finished water with no ammonium sulfate or nitrifying bacterium added was the negative control. Free ammonia controls on each sample were performed, where the samples were pH adjusted and inoculated with *N. europaea* to prove sufficient free ammonia existed at each site to support growth of nitrifying bacteria. The free ammonia controls were used to rule out free ammonia deficiency as an inhibiting factor for nitrifying bacterial growth.

The tubes were incubated at room temperature. To test for nitrite, aliquots of the samples were added to wells of a microtiter tray, and the nitrite-detecting reagents—N,N-dimethyl-alpha-naphthylamine and sulfanilic acid—were added (Figure 1).

By the 12th day of incubation, all five test wells turned pink, indicating nitrification by bacteria. This confirmed nitrifying bacteria were present in the distribution system water, apparently subsisting with no significant activity as indicated by the absence of nitrite. The positive growth control sample turned pink, indicating the presence of the added *N. europaea*, but the negative growth control sample showed no nitrite production. The free ammonia controls all turned positive for nitrite, indicating there was sufficient free ammonia in the distribution water to support growth (Figure 2).

To quantify nitrifying bacteria present in the distribution system, each sample was cultured by adding 0.1, 1.0, and 10.0 mL of water to a total volume of 10 mL of ammonium-sulfate-enriched

water, creating an ammonia nitrogen concentration of 0.5 mg/L. Growth of nitrifying bacteria was detected at site three, which had a concentration of 1-9 cells/mL. Growth was detected at sites one and four, having concentrations of 0.1-0.9 cell/mL. No growth was detected at sites two and five, corresponding to a concentration of <0.1 cell/mL. Relative to average heterotrophic bacterial counts in the DMWW system (23 colony-forming units/mL), the number of nitrifying bacteria was low (Figure 3).

Subsequent samples from the same sites were cultured for nitrifying bacteria in January, June, August, October, and November to determine if there was seasonal variation in the incidence of nitrifying bacteria present in the system. Despite water temperature ranges from the mid-40°F to mid-70°F range, there was no distinct difference in the number of nitrifying bacteria recovered.

Figure 1. Test Wells

To test for nitrite, aliquots of the samples were added to wells of a microtiter tray, along with nitrite detecting reagents.



Treatment

It's reasonable to extrapolate from this study that supplying the distribution system with relatively high pH water will inhibit nitrifying bacteria.

BIOFILM STUDY

Published literature has shown that planktonic, or individually suspended, bacteria in distribution water aren't necessarily representative of a system's overall bacterial colonization. Further, bacteria bound in biofilms throughout a system can amount to many times that of planktonic cells.

To determine if biofilms were a problem in the rural system, samples from a remote pumping station were collected. A sample of tap water was collected along with a swab sample from the inside of a 3-in.-diameter pipe. If significant biofilm existed at the site, DMWW personnel expected the swab sample to yield a higher bacterial count than the tap water sample.

Triplicate sets of 1-mL, 0.10-mL, and 0.01-mL volumes of the tap water were brought to 5 mL using filter effluent water with 0.5 mg/L NH₃-N added as ammonium sulfate. The swab was rinsed in 4 mL of sterile water collected at the same site, and this water was set up in identical fashion.

After 21 days of incubation with periodic testing throughout, the tap water experienced nitrification in all three 1-mL sample tubes. No nitrite was detected at the higher dilutions, indicating a bacterial count of 1–9 cells/mL (Figure 4). The swab water experienced nitrification in one of the three 1-mL sample tubes.

Because there was no greater recovery of bacteria isolated from the swabbed pipe interior, DMWW personnel concluded that there was no biofilm at this location. Although it's possible that distant biofilms were seeding the main, it seems more likely that the bacteria recovered were primarily planktonic in nature.

ANALYSIS

It's reasonable to extrapolate from this study that supplying the distribution system with relatively high pH water will inhibit nitrifying bacteria. This conclusion is further supported by *Bergey's Manual of Determinative Bacteriology*,

Figure 2. Positive Results

All five test wells turned pink by the 12th day of incubation, indicating the presence of nitrifying bacteria in the distribution system.



Figure 3. Quantifying the Nitrifying Bacteria

No growth was detected at sites two and five, corresponding to a concentration of <0.1 cell/mL.

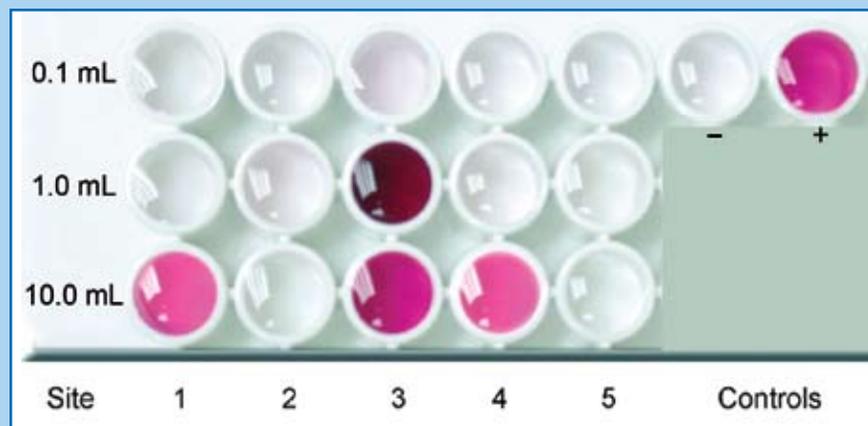
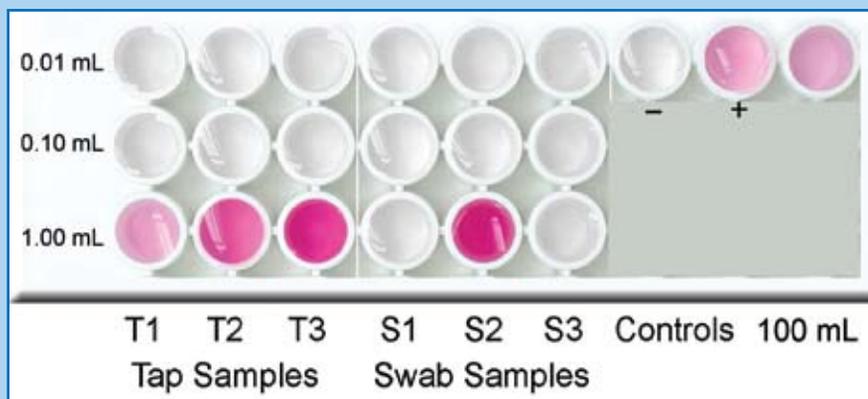


Figure 4. Planktonic Bacteria vs. Biofilm Bacteria

The tap water experienced nitrification in all three 1-mL sample tubes, whereas the swab water experienced nitrification in one of the three tubes.



which characterizes the entire group of ammonia-oxidizing bacteria as having an optimum pH below 8.0. So it's likely that an annual switch to chlorine isn't necessary in a distribution system maintained

at pH levels greater than 8.5. This has cost benefits for DMWW and prevents water quality changes associated with free chlorine-chloramination conversion that may be detected by customers. ❧