# Southeast Polk Rural Water North System Chloramination Biofilm Study

## *Spring Study*

**Dennis Hill - Microbiologist - Des Moines Water Works – June 2007**

**Des Moines Water Works (DMWW) began chloramination of the Southeast Polk Rural Water North (SEPO-N) distribution system early in 2006, because of elevated trihalomethane concentrations that occurred because of the use of free chlorine.**

**It is known that excess ammonia in a chloraminated water system can promote biofilm growth. Ammonia is a nutrient for nitrifying bacteria such as *Nitrosomonas* and others. *Nitrosomonas* biofilms can generate nitrite and deplete disinfectant.**

**In winter nitrification rates are slow in the cold distribution water, whereas accelerated nitrification is possible in the warmer distribution water of spring and summer.**

**In May, 2007, the Southeast Polk Rural Water distribution water increased in temperature from its winter readings in the 40's °F, to the lower 60's °F, which was sufficiently warm to allow the growth of nitrifying bacteria. As a follow-up to the winter SEPO-N study, samples were collected with the primary objective of determining the presence of nitrifying bacteria, which would indicate the potential for their growth if conditions became favorable. No nitrite had been detected in the SEPO-N distribution system using chemical analysis, suggesting that no significant nitrification of the chloramines has been occurring.**

**In a recent study by Terry Webster and the author, pH values above 8.5 were inhibitory for *Nitrosomonas europaea*.**

**Method:**

Five 100ml water samples were collected from the designated sampling sites used routinely for monthly coliform bacterial testing. These taps are well-maintained and periodically flushed, making them ideal for proper sample collection.

One milliliter of each sample was cultured for heterotrophic bacteria, using Standard Methods medium. The remaining 100ml of each sample were used as culture medium for the possible detection of distribution nitrifying bacteria.

A minimal amount of sodium thioglycolate was added to remove any residual chlorine, which with time could possibly inhibit growth of bacteria. Additional ammonium sulfate was added to increase the total amount of ammonia to

approximately 5mg/L. This was to ensure that enough ammonium would be available to support growth.

The initial pH of each sample was taken and recorded. This was repeated after incubation periods of four days and eleven days. In addition, the presence of nitrite was assessed after these incubation periods. This was done by using two reagents. The first was sulfanilic acid, used to lower the pH. The second was N,N-dimethyl alpha-naphthylamine, which produced a pink color from any existing nitrite in the sample.

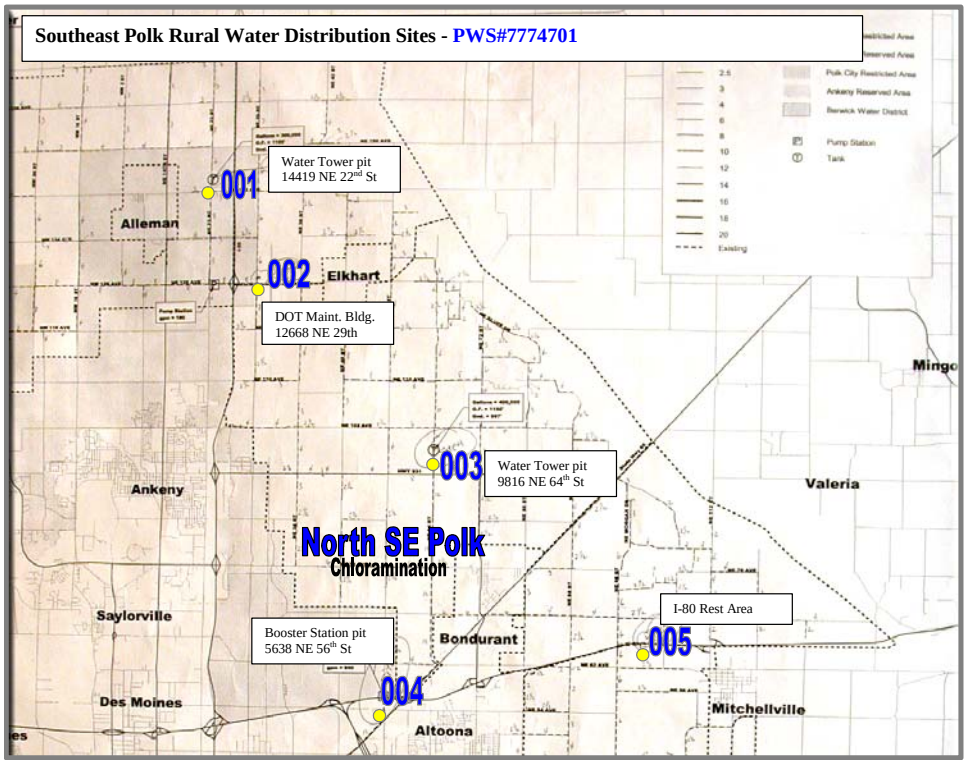
A duplicate set of samples was included. This second set was handled identically to the first, except the pH was adjusted down to approximately 7, to allow pH sensitive nitrifying bacteria to grow if they were present.

100ml Sample Bottles with added Ammonia

A positive control for each set of samples was made using a composite of each sample. Ammonium sulfate and the nitrifying bacterium, *Nitrosomonas europaea* were added to each. Two negative growth controls were comprised of DMWW finished water with no ammonium sulfate or bacterium added.

The sample sites were:

Sample site #1: Alleman Water Tower Pit, 14419 NE 22nd Street
Sample site #2: DOT Maintenance Bldg., 12668 NE 29th Street
Sample site #3: Water Tower Pit, 9816 NE 64th Street
Sample site #4: Booster Station Pit, 5638 NE 56th Street
Sample site #5: I-80 Rest Area (North)



The Standard Methods agar plates grew *Sphingomonas* and *Mycobacterium*. Both bacteria grew in similar numbers as in the winter study.

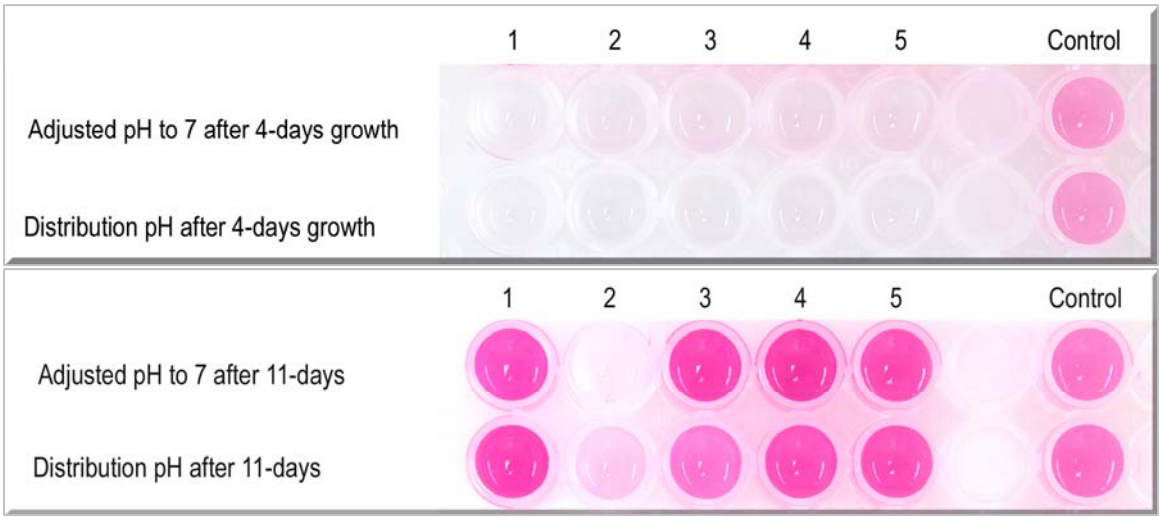
| Site | Nitrite | Number Bacterial Colonies | Bacteria Isolated |
|---|---|---|---|
| **Site #1** | None detected | 18/ml | *Sphingomonas*<br>*Mycobacterium* |
| **Site #2** | None detected | 0/ml | None |
| **Site #3** | None detected | 5/ml | *Sphingomonas*<br>*Mycobacterium* |
| **Site #4** | None detected | 132/ml | *Sphingomonas*<br>*Mycobacterium* |
| **Site #5** | None detected | 6/ml | *Sphingomonas*<br>*Mycobacterium* |

The initial pH values of the *first sample set* where the pH was not altered were all close to 9.1, except the sample from the Alleman Water Tower pit, which had a pH of 8.6.

After *four days incubation* at room temperature (approximately 72 °F), the *first sample set's* pH values dropped to approximately 8.0, except that of the Alleman Water Tower pit sample, which decreased to 7.5.

> pH decreases in distribution water as it is being studied in the laboratory. Distribution system water pH does trend down after leaving the plant, but the laboratory sample decreases more rapidly, especially when the sample is exposed to air, which is necessary for proper culture conditions. This does not interfere with the growth and detection of nitrifying bacteria.

At the four day incubation mark, an aliquot of each sample of both sets was placed into a separate tube for the determination of the presence of nitrite. No pink color developed in any of the test tubes of either set, indicating the absence of nitrite and therefore the absence of nitrification. At this stage there was no evidence of the presence of nitrifying bacteria in the distribution water. The positive growth control sample turned pink, expressing the presence of the added *Nitrosomonas europaea*, whereas the negative growth control sample showed no nitrite production.



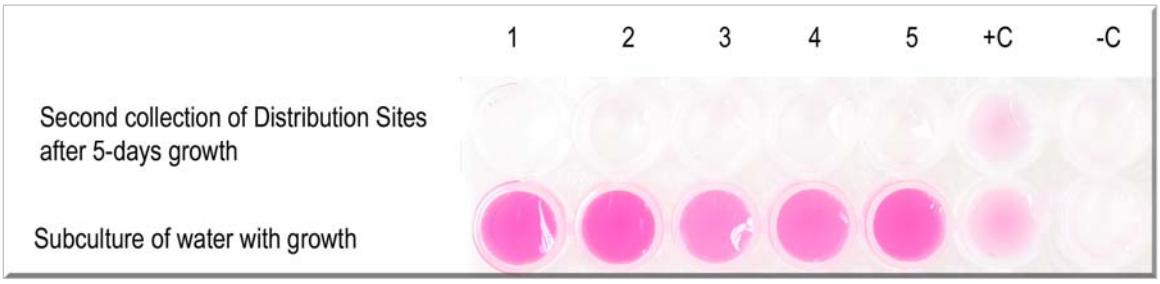
After *eleven days incubation*, the pH values all decreased to values ranging from 6.7 to 7.6.

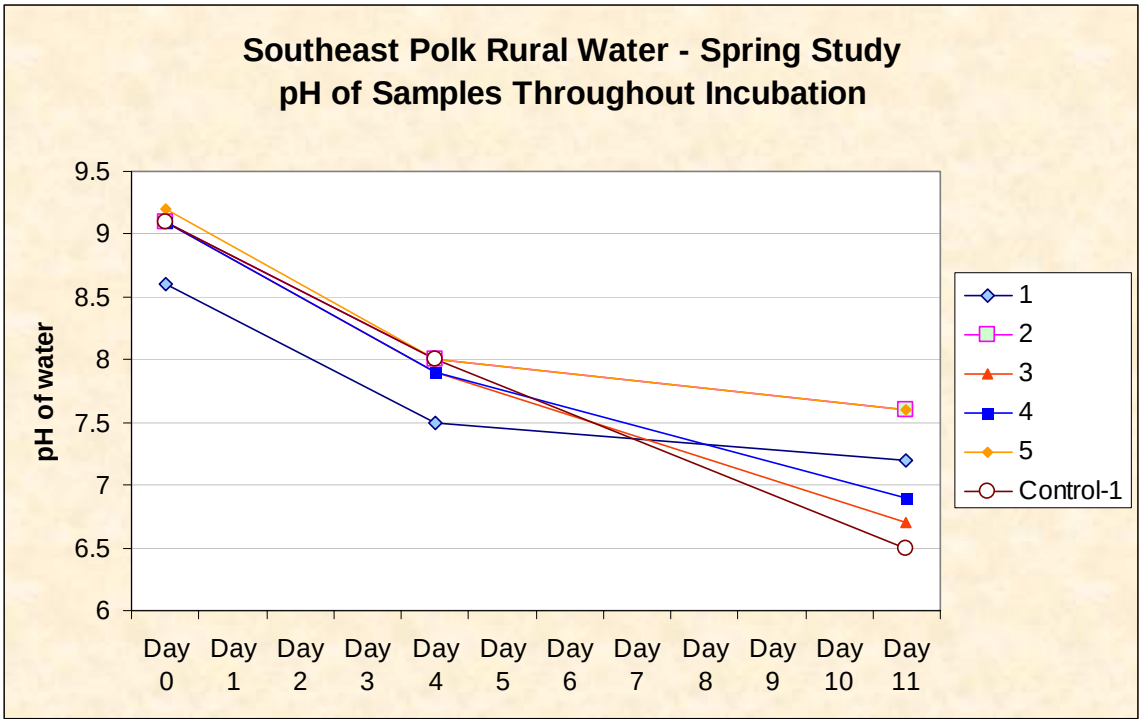
An aliquot of each sample of the *first set* that was tested for nitrite turned pink, at this stage revealing nitrite production by apparent nitrifying bacteria. The reaction of sample #2 was weak, suggesting possibly a minimal initial inoculum or presence of nitrifying bacteria.

All aliquots of the *second set* expressed the presence of nitrite, except sample #2 of that set, suggesting a bacterial quantity at that site that was too low for detection using a 100ml sample.

To determine if the distribution nitrifying bacteria were minimal in initial numbers or if they were fastidious growers, each of which could account for the delayed nitrite production, a set of tubes of fresh distribution water was inoculated with water from the old set containing growth of nitrifying bacteria. A second set of fresh distribution water was incubated in parallel without a subculture inoculum.

Within five days, a strong nitrite reaction occurred in all five tubes with the subcultured inoculum of the nitrifying bacteria, indicating that the organism is robust, but must presently exist in low numbers in the distribution. The control set had no nitrification detected at five days.



Cloudiness was observed in the positive control bottles, which is an unusual phenomenon for nitrifying bacteria, because of their relatively light growth in contrast to heterotrophic bacteria. These control samples were subcultured to Standard Methods agar to determine the presence or absence of the suspected heterotrophic bacteria. Two species of heterotrophs were recovered. They each were tested for their ability to denitrify, and were found lacking in the trait. They were thus considered insignificant contaminants, except for their ability to decrease the pH due to acid end-product production. Their origin was likely from the ATCC stock culture broth of the control *Nitrosomonas europaea*.

**Summary:**

Evidence of bacterial nitrifying *activity* in the Southeast Polk Rural Water District North system was not found in this spring study. However, evidence of their *existence* was detected by their eventual growth in ammonium-enriched distribution samples where pH in the laboratory dropped to 8.5 or lower. This indicates that maintenance of a relatively high pH in our distribution system water will be important for the prevention of biofilm development.

As is common with other bacteria in other environs, if their key nutrient is present and other growth conditions are favorable, then they will very likely be present. We are supplying nitrifying bacteria their key nutrient ammonia, therefore we must work to keep growth conditions, such as a moderately low pH, from evolving.

An alternate option is to be unconcerned with biofilm development by nitrifying bacteria. By choosing this latter approach, we would sacrifice some control of water quality in the distribution system, since the proved presence of nitrifying bacteria means a significant biofilm would likely occur. The warm water of summer and fall traditionally maintains lower levels of chlorine or chloramine residuals. Both warmth and low chlorine is more conducive to biofilm growth. An increase in water demand during these warm months will help create a favorable turn-over of water in the system, but the development of a biofilm at this time would be inopportune. To eliminate biofilms by chlorinating with free chlorine, may prove difficult when weather is warm and chlorine residuals are hard to maintain. This latter scenario would also create less consistent water quality conditions for the Southeast Polk Rural Water customers.

With future studies, it might be possible to enumerate planktonic nitrifying bacteria in the SEPO-N distribution system, by performing dilutions on freshly collected samples. This might help us determine the progression of growth of these organisms at any one site in the system.