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Bacterial Nitrification in Chloraminated Water Supplies

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Nitrifying bacteria were detected in 64% of samples collected from five chloraminated water supplies in South Australia and in 20.7% of samples that contained more than 5.0 mg of monochloramine per liter. Laboratory experiments confirmed that nitrifying bacteria are relatively resistant to the disinfectant. Increased numbers of the bacteria were associated with accelerated decays of monochloramine within distribution systems. The combination of increased concentrations of oxidized nitrogen with decreased total chlorine residuals can be used as a rapid indicator of bacterial nitrification.

The growth of nitrifying bacteria has been identified as a potential problem in chloraminated water supplies (13, 14). These bacteria derive energy by the oxidation of ammonia to nitrite or of nitrite to nitrate (11). The oxidation of ammonia can be undertaken by several genera, including *Nitrosomonas*, *Nitrosolobus*, *Nitrosococcus*, *Nitrosovibrio*, and *Nitrosospira*, while oxidation of nitrite in water is undertaken predominantly by members of the genera *Nitrobacter* and *Nitrococcus*.

Bacterial nitrification can lead to rapid decays of chloramines, but the mechanism by which this occurs has not been established (14). It has been suggested that the production of nitrite could accelerate chloramine decay, particularly in the presence of bromide (9), or that ammonia oxidation could shift the equilibrium of monochloramine formation so that as free ammonia is metabolized, monochloramine is hydrolyzed (14).

In South Australia water supplies, bacterial nitrification has been associated with accelerated decays of chloramines, which have led to losses of disinfectant from sections of water distribution systems. Once established, large populations of nitrifying bacteria appear to act as barriers to the redistribution of chloramines throughout affected systems.

The purposes of this investigation were to examine (i) the distribution of nitrifying bacteria in South Australia water supplies, (ii) the sensitivity of the bacteria to disinfection by monochloramine, and (iii) any association between numbers of nitrifying bacteria and other selected parameters, including temperature, standard plate counts, oxidized nitrogen (nitrate plus nitrite), and total chlorine. The aim of the latter purpose was to identify early indicators of the proliferation of the bacteria, since the direct method most commonly used to enumerate nitrifying bacteria is relatively inefficient and takes at least 3 to 4 weeks to provide a result (2, 8).

Samples were collected from five distribution systems by standard procedures (16). The systems supply chloraminated water to predominantly rural areas; two distribute filtered water, and three distribute unfiltered water. The major pipelines incorporated in the systems range from about 40 to 370 km in length. Oxidized nitrogen concentrations were determined by an automated cadmium reduction method (1) with a SKALAR automatic flow analyzer (sans plus system). Total chlorine and monochloramine residuals were determined by titration with ferrous ammonium sulfate with *N*,*N*-diethyl-*p*-phenylenediamine as a colorimetric indicator (1).

Nitrifying bacteria were enumerated by using a modification of the most-probable-number procedure described by Wolfe et al. (13) and incorporating the medium of Skinner and Walker (8). Up to four sets of five tubes each were used, and these were inoculated with 1-, 0.1-, 0.01-, and 0.001-ml volumes of the sample to be tested. The 1-ml volumes were inoculated into 9 ml of medium, while the smaller volumes were inoculated into 1 ml of medium. Where required, dilutions were prepared in the growth medium. After inoculation, tubes were incubated in the dark for 28 days at 30°C. A 10-ml volume of sample was frozen and stored. After incubation, the tubes were examined for change of color from red to yellow; when color changes were observed in any tube, all tubes in that set were then examined for the production of nitrite by mixing 5 drops from the most-probable-number tube with 1 drop each of sulfanilic acid solution (0.8 g of sulfanilic acid in 100 ml of 5 M acetic acid) and *N*,*N*-dimethyl-naphthylamine solution (1.2 ml of *N*,*N*-dimethyl-naphthylamine in 100 ml of 5 M acetic acid). Production of dark pink to red color in 5 min at room temperature indicated the presence of nitrite. A small amount of zinc powder was added to tubes that were negative for nitrite. The zinc reduces any nitrate that may be present to nitrite. The production of a pink to red color in 15 min at room temperature indicated the presence of nitrate. The detection of either nitrite or nitrate was regarded as indicating the presence of nitrifying bacteria. As a control, the same tests were performed by using 5 drops of water from the original sample. Numbers of bacteria were determined with a standard most-probable-number table (16). The limit of detection of the assay was 0.2 organisms per ml.

Between 1988 and 1990, 1,184 samples were analyzed, of which 758 (64%) contained nitrifying bacteria (Table 1). The frequency of detection and median number of bacteria decreased as the total chlorine residual increased, but nitrifying bacteria were still detected in 20.7% of samples that contained more than 5.0 mg of total chlorine per liter when collected. Surveys of individual systems showed that there is an association between distance from the chloramine dosing station and the frequency of detection of the bacteria. Typical results from a survey performed for the Yorke Peninsula system in 1988 and 1989 are shown in Table 2.

The sensitivities of natural populations of nitrifying bacteria to inactivation by monochloramine were measured in laboratory experiments. Experiments were performed at 30°C and pH 8.0. These conditions were chosen to be consistent with optimal growth requirements for nitrifying bacteria (11) and with those found in local chloraminated

TABLE 1. Frequency of detection of nitrifying bacteria in chloraminated water

Total chlorine residual (mg/liter)	No. of samples		Median no. of nitrifying bacteria/ml
	Tested	Containing nitrifying bacteria (%)	
0.1–0.2	343	302 (88.0)	130
0.3–1.0	156	110 (70.5)	4.1
1.1–2.0	215	137 (63.7)	2.0
2.1–3.0	182	103 (56.6)	0.5
3.1–4.0	134	61 (45.5)	<0.2
4.1–5.0	62	26 (41.9)	<0.2
>5.0	92	19 (20.7)	<0.2
Total	1,184	758 (64.0)	2.0

supplies (3). Five-hundred-milliliter volumes of reservoir water containing a natural population of nitrifying bacteria (predominantly ammonia oxidizers) were treated with between 1 and 5 mg of monochloramine per liter, and the survival of the bacteria was monitored. Immediately before the addition of monochloramine, a 10-ml subsample was collected. Further samples were collected after 15, 30, 60, 90, 120, 180, and 240 min and then at 120-min intervals up to 840 min, with a final sample at 1,440 min. These subsamples were mixed with 0.1 ml of 1% (wt/vol) sodium thiosulfate to neutralize the monochloramine. Nitrifying bacteria were enumerated as described above. Monochloramine residuals were monitored through the course of each experiment. Times required to inactivate 99% of bacterial populations were determined by plotting log percentage survival against time (in minutes). After initial lag periods, these plots produced straight lines with correlation coefficients (r^2) in the range of 0.92 to 0.99, as determined by regression analysis.

The results shown in Fig. 1 are expressed in terms of the formula of Watson (10) in which $k = C^n \cdot t$, where k is a constant, C is the concentration of disinfectant, t is the time taken to inactivate 99% (in this case) of the original inoculum, and n (the slope of the line) is the coefficient of dilution. The concentrations of disinfectant expressed in Fig. 1 are the averages of the initial doses and the concentrations at the 99% inactivation time. The initial doses were 1.0, 2.2, 3.2, 4.1, and 5.2 mg/liter while the average doses were 0.6, 1.85, 3.05, 4.05, and 5.2 mg/liter, respectively.

The experiments confirmed the relative resistance of the

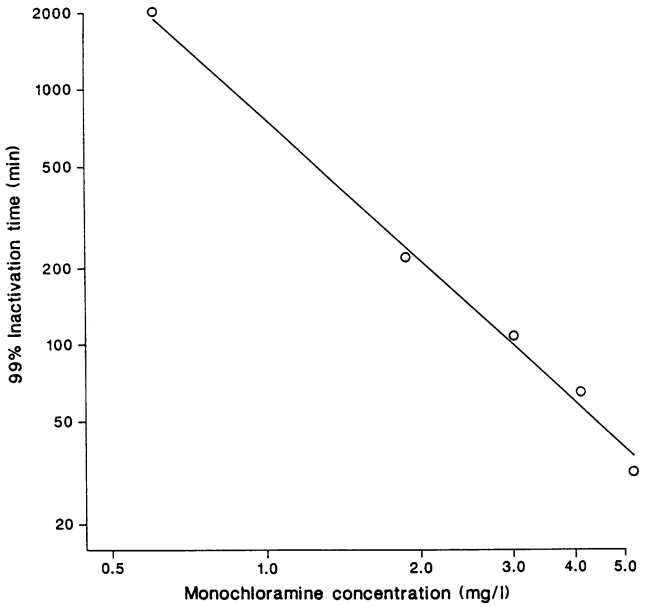

FIG. 1. Inactivation of nitrifying bacteria by monochloramine. The line of best fit was determined by regression analysis.

bacteria to disinfection by monochloramine. On the basis of the results, inactivation of 99% of a natural population of nitrifying bacteria with an average dose of 1 mg of monochloramine per liter would take 760 min. In contrast Wolfe et al. (13) found that 99% inactivation of laboratory-cultured bacteria was achieved in 3 to 33 min of treatment with 1.0 mg of monochloramine per liter, which, as indicated, was not consistent with the prevalence of nitrifying bacteria in chloraminated supplies. Differences in experimental design could explain the disparity between the two sets of laboratory results. Wolfe et al. (13) used demand-free water and cultured bacteria. The latter could be significant, as it has been found for both *Flavobacterium* spp. and *Legionella pneumophila* that culturing reduces resistance to disinfection (6, 15).

The slope of the line (n) in Fig. 1 is 1.85, which exceeds the ideal n value of 1 (7, 12) and means that the $C \cdot t_{99}$ product is not constant. It also suggests that the concentration of disinfectant is more important than time for the inactivation of nitrifying bacteria (7).

The relative resistance to monochloramine does not fully

TABLE 2. Occurrence of nitrifying bacteria in the Yorke Peninsula distribution system, 1988 and 1989

Location	Distance from chloraminator (km)	No. of samples	No. positive (%)	Median no. of bacteria/ml	Median chloramine residual (mg/liter)
Paskeville Reservoir		21	19 (90.5)	2.5	<0.1
Paskeville (after chloramination)	1.5	12	1 (8.3)	<0.2	6.4
Arthurton	26.7	20	0 (0)	<0.2	3.9
Maitland	41.6	20	1 (5.0)	<0.2	5.6[a]
Mount Rat	82.8	15	8 (53.3)	0.2	2.2
Minlaton	100.6	21	7 (33.3)	<0.2	2.5
Minlacowie	110.0	20	7 (35.0)	<0.2	2.3
Pt. Vincent[b]	124.8	16	10 (62.5)	0.9	2.5
Stansbury[b]	129.1	14	14 (100)	3.5	2.2
Yorketown	129.1	15	10 (66.7)	1.3	2.3
Edithburgh	143.2	17	15 (88.2)	3.5	2.0

[a] Supplementary chloramination occasionally applied at Maitland.
[b] On branch main starting at Minlaton.

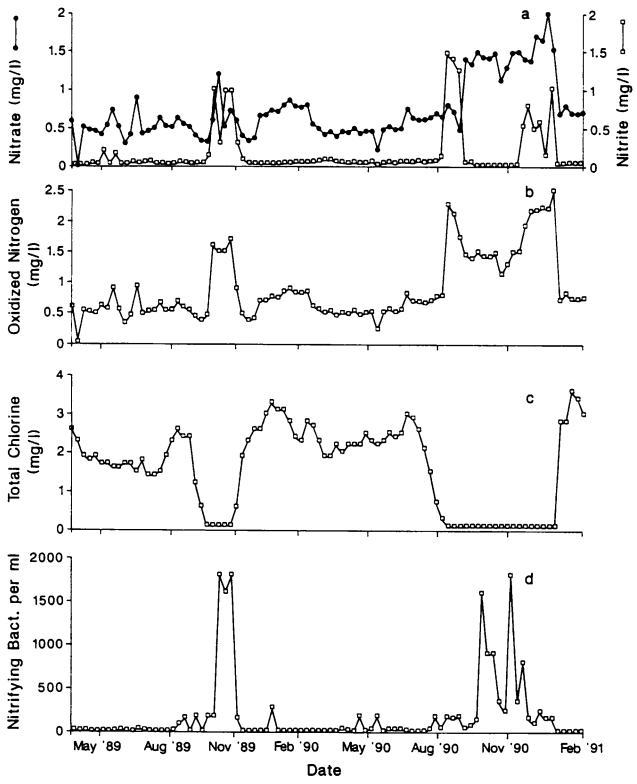

FIG. 2. Relationship between nitrite and nitrate concentrations (a), oxidized nitrogen concentrations (b), total chlorine residuals (c), and numbers of nitrifying bacteria (d) from samples collected between May 1989 and February 1991 from Edithburgh.

explain the prevalence of nitrifying bacteria in the water supplies. A second mechanism that may contribute is survival or growth in biofilms. Nitrifying bacteria can grow in aggregates and attach to surfaces (11) and have been detected in large numbers in sediments (4). If the bacteria exist in biofilms, then the organisms detected from samples containing high total chlorine residuals may have been dislodged shortly before or during sampling. The frequent detection of nitrifying bacteria at the ends of distribution systems could be due to a combination of decreased water flows, which would favor the formation of biofilms, and lower chloramine residuals.

In South Australia water supplies, most problems associated with nitrifying bacteria have occurred at the ends of distribution systems. Typical results are shown in Fig. 2. Water samples were collected every week from the town of Edithburgh, which is located near the end of the chloraminated Yorke Peninsula system, and analyzed for oxidized nitrogen, nitrate, nitrite, total chlorine, and nitrifying bacteria. In 1989 and 1990 there were two periods in which chloramine decay within the Yorke Peninsula distribution system accelerated to the point that the total chlorine residual at Edithburgh decreased from 1.5 to 2.5 mg/liter to <0.1 mg/liter (Fig. 2). On both occasions there was an associated increase in bacterial nitrification. The numbers of nitrifying bacteria and concentrations of oxidized nitrogen, nitrite, and nitrate all increased (Fig. 2). Typically, nitrite concentrations increase relatively quickly but are not always sustained, particularly during longer periods of decay. Between August and December 1990 there were two peaks of nitrite concentration.

The relationship between oxidized nitrogen concentrations, total chlorine residuals, and the presence of nitrifying bacteria was confirmed by using stepwise multiple logistic regression (5). Results from 1,001 samples were analyzed; oxidized nitrogen, total chlorine, standard plate counts, and temperature were investigated as potential predictors of the presence of nitrifying bacteria. The logistic analysis selected oxidized nitrogen and total chlorine as significant predictors, whereas standard plate counts and temperature were not statistically significant. Presence was defined as being >1 organism per ml; when presence was defined as being >10 or >100 organisms per ml, the predictive nature of the logistic model was not as strong.

There have been a number of episodes of bacterial nitrification in South Australia water supplies. On many occasions it was not established whether bacterial nitrification was the primary cause of the accelerated decay or whether growth of the bacteria followed as a consequence of the decay. Both alternatives are credible, and it is possible that both have occurred at different times. If increased numbers of the bacteria are a primary cause of accelerated decay, the question then becomes what event(s) triggers the multiplication of the bacteria? It has been suggested that in Southern California, water temperature is critical and that ammonia-oxidizing bacteria grow only above 16 to 18°C (13). However, analysis of results from South Australia water supplies using multiple logistic regression did not identify temperature as a predictor for the presence of the bacteria. Further, nitrifying bacteria were detected in water with temperatures ranging from 10 to 34°C, and while most episodes of accelerated decay have occurred in late spring and summer, there have been episodes in winter. Spearman's rank correlation coefficients calculated from the data set mentioned above also failed to support any significant association between the two parameters ($r_s = 0.01$, $P > 0.5$). Both oxidized nitrogen ($r_s = 0.47$, $P < 0.001$) and total chlorine ($r_s = 0.5$, $P < 0.001$) did show a degree of correlation with the numbers of bacteria.

On the basis of the statistical analyses, current practice in South Australia is to monitor trends in total chlorine residuals and in the concentration of oxidized nitrogen at individual locations near the ends of each distribution system. An increase in oxidized nitrogen combined with a decrease in total chlorine can be used as an early indication of bacterial nitrification, eliminating the need to wait for the results of the bacterial analyses. Rapid diagnosis enables appropriate remedial action to be undertaken quickly to limit the extent and effect of nitrification. Once established, removal of large populations of nitrifying bacteria has often required the use of free chlorine.

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