Is Overstabilization of Stabilized Bromine a Significant Concern?

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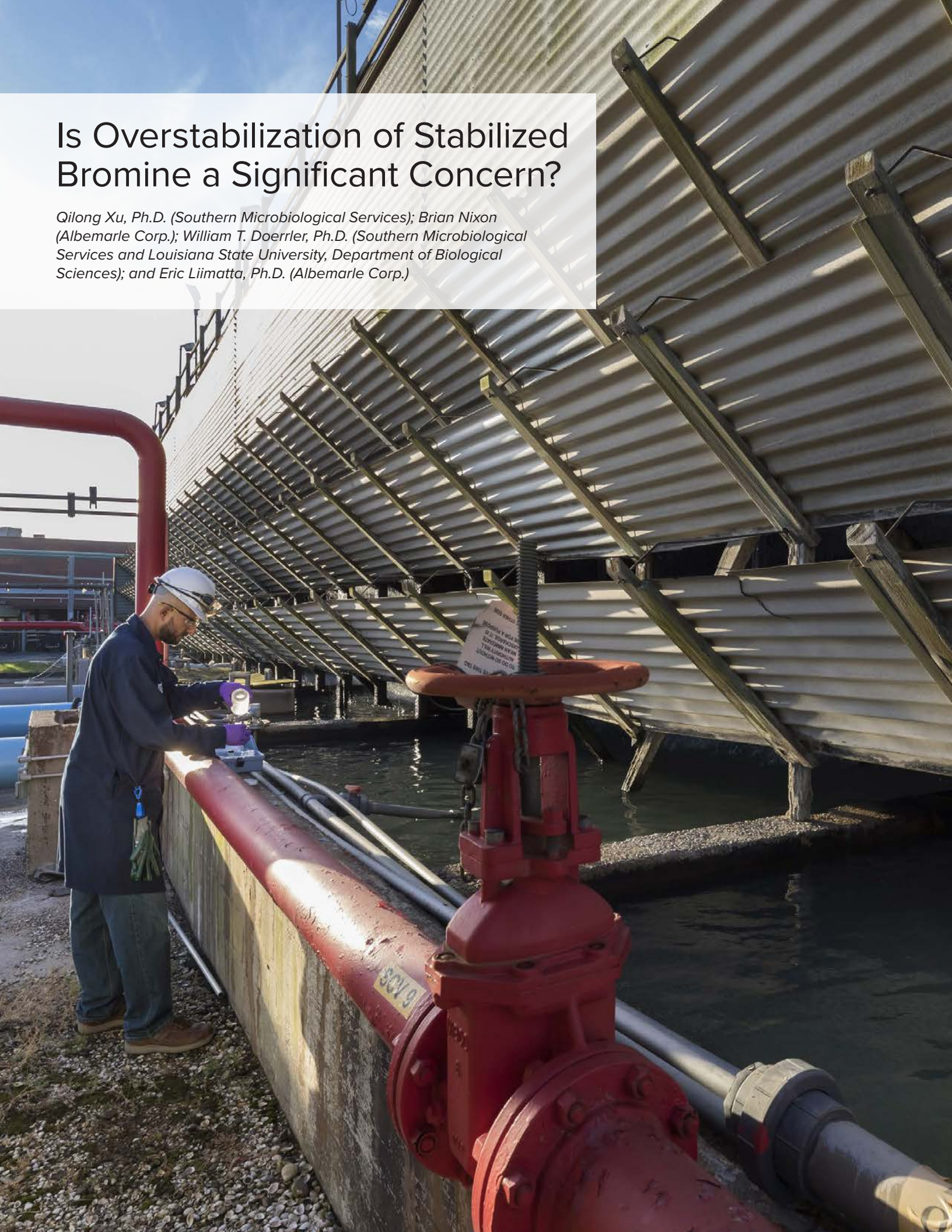


Abstract

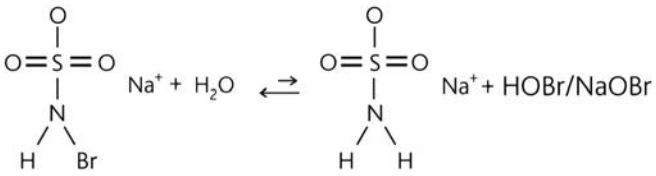
The buildup of stabilizer in a cooling tower over time and the possibility of diminished biocidal efficacy is a concern, especially in cooling towers with high cycles of concentration. There is certainly some precedent for this concern as chlorine in swimming pools can be overstabilized by cyanuric acid. It has been speculated that having a certain level of stabilizer in a cooling tower would indicate that the biocide is overstabilized. However, we are unaware of any studies examining overstabilization in any detail. In this article, efficacy studies were done as a function of sulfamate levels to determine the effect on both planktonic organisms and biofilms. Lastly, samples were taken from cooling towers treated with stabilized bromine so that the measured sulfamate levels could be compared to the biocidal performance. In all of these cases, high levels of sulfamate (>100 parts per million [ppm]) did not significantly hinder the biocidal performance. In the biofilm studies, sulfamate levels greater than 150 ppm were required to observe degradation in biocide performance.

Introduction

The commercialization of stabilized liquid bromine in the late 1990s has provided a powerful new tool in the arsenal to control bacteria in a cooling tower. This material uses sulfamic acid as the stabilizer. Stabilized liquid bromine is a stable, easy-to-feed, single-component biocide, which has significantly enhanced efficacy over chlorinated biocides at the alkaline pH values typically observed in the cooling tower. Stabilized bromine was so successful that it and bleach are now the most widely used oxidizing biocides in industrial water treatment.

Much of this enhanced storage stability is because of the addition of a stabilizer. While there are several possible stabilizer options, sulfamic acid is currently the most widely used. Figure 1 demonstrates how the active biocide is generated when added to water.

Figure 1: The hydrolysis of stabilized bromine in water to prepare the active biocide.



Sporadic anecdotal reports from the field, however, indicate that the halogen residual that once provided the desired level of microbiological control was no longer providing the same level of control, even though there was no apparent change in the system. One possible explanation for this observation is overstabilization. Overstabilization may occur when there is a large excess of sulfamic acid stabilizer present, and the hypobromous acid becomes very tightly bound and thus less available to kill bacteria. As one might expect, overstabilization would be more likely to occur in cooling towers with high cycles of concentration.

Despite the concern about overstabilization, and the long period of commercial use of stabilized bromine products, we are unaware of any published data on this topic. Some members of the industrial water treatment community have suggested that if the cooling tower contains above a certain ppm level of sulfamate, the cooling tower is overstabilized. One biocide supplier has suggested that if the ratio of sulfamate to available bromine in the water is over 20, the cooling tower is overstabilized. While these points of view may be accurate, we are unaware of any data supporting these observations.

The authors' view on this issue is a little more nuanced. Our belief is that the level of sulfamate in the water required for overstabilization will depend on conditions in the cooling tower. Thus, there is no single number for the sulfamate level that one can measure and definitely assert whether a cooling tower is having issues with overstabilization. In this article, we will present planktonic data, biofilm data, and real-world data on the effects of sulfamate levels on biocidal efficacy to support this view.

Materials and Methods

- Experimental materials used in the studies included a stabilized bromine biocide.[A] All other chemicals and growth media were purchased from Thermo-Fisher. Dip slides were from Solar Biologicals Inc.

- Synthetic water (SW) was prepared by adding 0.22 grams (g) of calcium chloride ($CaCl_2$) and 0.168 g of sodium bicarbonate ($NaHCO_3$) to 1 liter (L) of deionized water (DW). The water had 150 ppm of alkalinity (as calcium carbonate [$CaCO_3$]), and 200 ppm Ca hardness (as calcium carbonate [$CaCO_3$]) and a pH of approximately 8.

- Before each use, *Escherichia coli* (ATCC BAA1427) or *Pseudomonas aeruginosa* PAO1 was cultured in tryptic soy broth (TSB) overnight and cultured again for 16 hours (h). The recultured bacteria were pelleted by centrifugation at 5,000 revolutions per minute (rpm) for 10 minutes (min) and washed once in phosphate-buffered saline (PBS, NaCl 8.0 g/L, potassium chloride [KCl] 0.2 g/L, disodium phosphate [Na_2HPO_4] 1.44 g/L, monopotassium phosphate [KH_2PO_4] 0.24 g/L) and resuspended in PBS to 8.5 log colony forming units per milliliter (CFU/mL).

- Halogen solutions or synthetic water (9 mL) and prepared bacteria (1 mL) was mixed and incubated at room temperature with 200 rpm orbital shaking. At 5 min, 30 min, 60 min, or 120 min, 1 mL from each mixture was transferred to 9 mL of 0.1% buffered peptone water containing 0.05% thiosulfate (BPWT) and mixed well. Each solution was tested in duplicate.

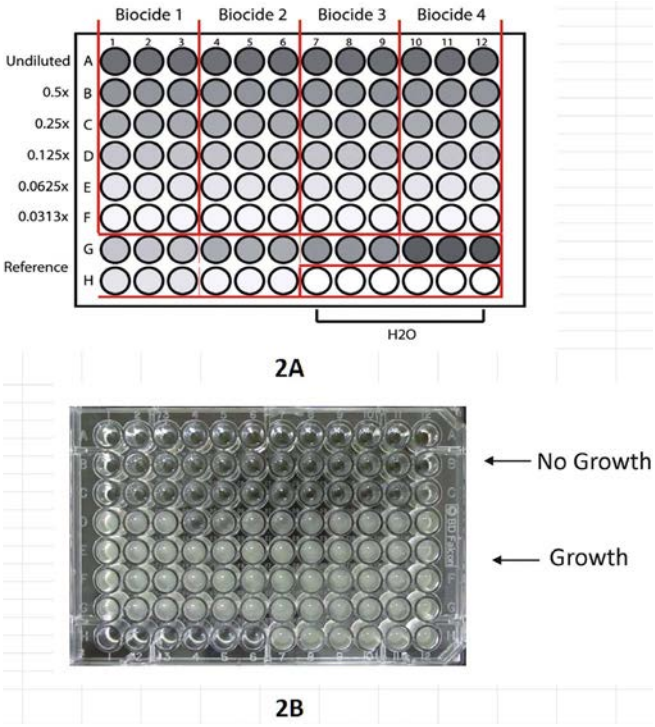
- The solutions from each treatment were 10x diluted in BPWT. Aerobic petri-film counts (APC, 3M) were used for the colony counts of *E. coli* and *P. aeruginosa*.

- *Pseudomonas aeruginosa* (ATCC 27853), which had been maintained on Luria Broth (LB) agar at 4 °C, was cultured in LB broth at 37 °C for 18 to 20 h. Cells were centrifuged at 5,000 rpm for 5 min, washed twice with phosphate-buffered saline (PBS, pH 7.4), and re-suspended in LB broth. Cell density was adjusted to 0.25 at the optical density 600 nanometers (nm) (OD600). To each well of 96-well plate (treated, Corning), 200 microliter (µL) of the resuspended solution was added. The plate was wrapped with a single layer of Parafilm and put in an autoclave tray. The plate was covered with 8 to 10 layers of warm, wet paper towels. The tray was covered with aluminum foil and incubated overnight (18 to 24 h) at 37 °C oscillated at 72 rpm.

- To avoid contamination, all treatments were performed in a biosafety hood. The growth media from the 24-h biofilm culture was removed from the 96-well plate by aspiration. The plate was washed twice with 200-µL sterile water and once with 220-µL sterile water. Biocides were serially diluted and were applied with 300-µL/well as triplicates per the matrix described in Figure 2A. The plate was incubated at room temperature for one hour without shaking. Biocides were removed and the wells were washed three times with sterile water by aspiration from highest to the lowest concentration (Figure 2A). 210 µL LB or sterile water was added. The plate was sealed with a layer of Parafilm and incubated overnight at 37 °C without shaking. The regrowth was visually read, based on the appearance of the wells (Figure 2B). The lowest concentration of biocide that did not allow bacterial regrowth (thus complete kill of the bacteria in the well) is called the minimum biofilm eradication concentration (MBEC).

- The sulfamate levels in the cooling water samples were measured by ion chromatography on a Thermo Scientific Dionex ICS-5000 5000 instrument using a potassium hydroxide (KOH) eluent generator and a conductivity detector for anions.

Figure 2: 2A (top) shows the measurement of biocidal efficacy against bacterial biofilm using a 96-well plate. After biofilm growth for 24 h, media is removed and wells washed with water. Biocides are applied in triplicate to Row A, followed by serial dilutions in Rows B-F as shown. Following incubation with stabilized bromine, wells are washed, growth media is added, and plates are incubated to allow detection of surviving biofilm. Untreated biofilms and media-only (cell-free) wells are included as controls (called reference in 2A). 2B (bottom) shows a 96-well plate after regrowth. The clear well indicates complete kill of the biofilm bacteria (no growth). Turbidity indicates incomplete kill of the biofilm bacteria (growth).



Results

Our first efforts to examine the effects of sulfamate levels on biocidal efficacy were with planktonic bacteria. These were time kill studies. Pure bacterial cultures were used for this work to ensure that if multiple tests were required, the test matrix would be the same for each test. Please note that the sulfamate level noted in the tables is in addition to the stabilizer that is already contained in the product. In Table A, the test bacteria are *E. coli*. It is very clear that the sulfamate levels greatly affect the efficacy at shorter contact times. However, at longer contact times (and certainly very reasonable contact times for a cooling tower) complete kill was achieved, even with 100 ppm additional sulfamate added.

Table A: *E. coli* Time Kill Study

	5 min	*30 min*	*60 min*	*120 min*
5 ppm	4.3	ND	ND	ND
5 ppm + 25 ppm SA	0.16	5.20	ND	ND
5 ppm + 50 ppm SA	0.02	6.02	ND	ND
5 ppm + 100 ppm SA	0.00	2.02	5.25	ND

Notes:
ND = not detected (complete kill) SA = Sulfamate
The initial titer was 8.36 log CFU/mL of bacteria. The solution pH is 8.0. The data in Table A represents log reduction.

To observe the effects of a very large excess of sulfamate (560 ppm), the study summarized in Table B was performed. In this case, the efficacy of the biocide is severely degraded. In this instance, the solution with the excess sulfamate shows less efficacy at 60 min than the solution with no excess stabilizer at 5-min contact time. This solution is clearly overstabilized.

Table B: *E. coli* Time Kill Study With 560 ppm Sulfamate

	5 min	*30 min*	*60 min*
5 ppm	2.29	ND	ND
5 ppm + 560 ppm SA	0.07	0.08	0.25

Notes:
ND = not detected (complete kill) SA = Sulfamate
The initial titer was 8.72 log CFU/mL of bacteria. The solution pH is 8.0. The data in Table B represents log reduction.

We repeated this experiment with a different bacterium to see if the behavior observed for *E. coli* was typical. For this next set of experiments, a bacteria commonly found in cooling towers (*P. aeruginosa)* was used. As shown in Table C, very similar behavior was observed. At short contact times, the addition of sulfamate reduces biocidal efficacy, but again, even with the addition of 100 ppm additional sulfamate, acceptable biocidal efficacy is achieved at longer contact times.

Table C: *Pseudomonas aeruginosa* Time Kill Study

	5 min	*30 min*	*60 min*	*120 min*
7.5 ppm	2.14	6.11	ND	ND
7.5 ppm + 25 ppm SA	0.03	2.77	5.36	6.76
7.5 ppm + 100 ppm SA	0.01	2.32	3.96	7.14
10 ppm	3.00	7.76	ND	ND
10 ppm + 25 ppm SA	0.1	5.33	7.76	ND
10 ppm + 100 ppm SA	.03	3.62	7.76	ND

Notes:
ND = not detected (complete kill) SA = Sulfamate
The initial titer was 8.91 logs CFU/mL. The solution pH is 8.0. The data in Table C represents log reduction.

The next set of experiments was on biofilm bacteria. This is a more challenging experiment, as biofilm are much more difficult to kill than planktonic bacteria. Again, the goal was to determine the effect of the sulfamate level on biocidal efficacy. The experiment involved a 96-well test system employing one-day-old *Pseudomonas aeruginosa* biofilm, and the biocidal contact time was one hour.

Please note that the *P. aeruginosa* strain used in the biofilm experiments is different than the strain used in the planktonic experiments. To increase the sensitivity of the experiment (minimize the difference between biocidal challenge concentrations), instead of just performing the standard serial dilution in each column of the 96-well plate, two different biocide concentrations were used and then serially diluted in each column. Figure 3A shows there is no difference in efficacy in this test for material with any added sulfamate to solutions with up to an additional 150 ppm of sulfamate. At all levels of added sulfamate tested, the MBEC was 6 ppm.

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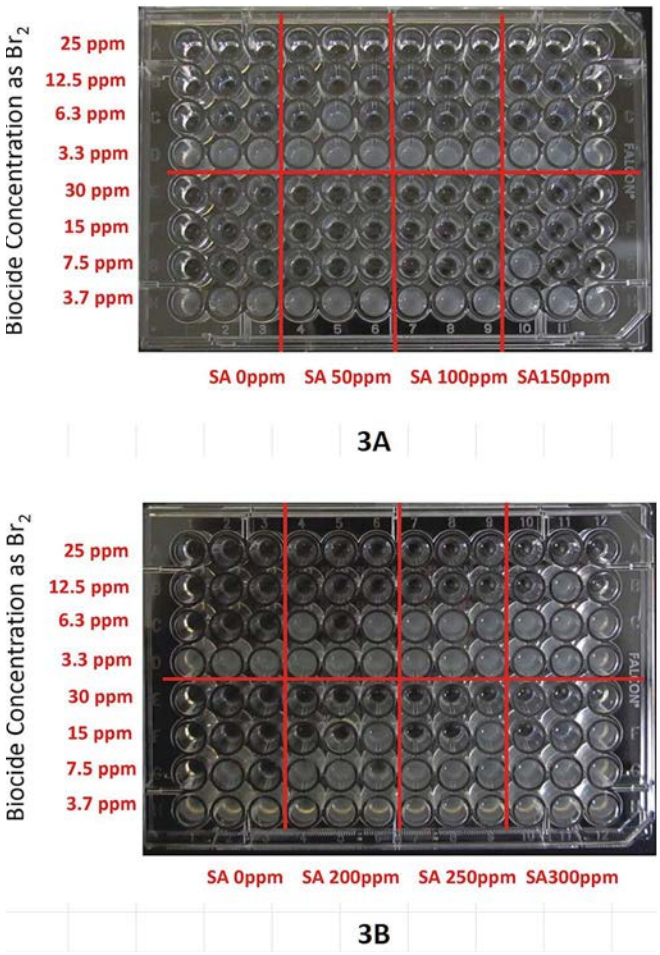
Figure 3: 3A (top): Effect of one-hour biocidal contact time with varying amounts of stabilizer against a one-day-old *Pseudomonas aeruginosa* biofilm. 3B (bottom): Cloudy wells indicate that the bacteria were not all killed by the biocide.



Since no efficacy differences were observed at up to 150 ppm added sulfamate, higher levels of sulfamate were tested. Figure 3B shows the results of that test. In this case, clear differences in efficacy were observed. Again, the biocide with no added sulfamate had an MBEC of 6 ppm. However, the biocide with 200 and 250 ppm of added sulfamate had an MBEC of 13 ppm, and the wells with 300 ppm sulfamate had an MEC of 25 ppm.

The next component of this study was to examine real-world samples. We wanted samples from cooling towers that were using stabilized bromine as a biocide and were maintaining good microbiological control. The sulfamate levels in these cooling towers would provide us with information on sulfamate levels that can exist in cooling towers that were under microbial control.

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Samples from five cooling towers at a chemical manufacturing plant were acquired and the sulfamate level measured. These cooling towers are slug-dosed daily with stabilized bromine, and the microbial level in the cooling water is controlled to 10^3 to 10^4 CFU/mL. The cycles of concentration are approximately seven for each tower. In addition, dip slides were sent with the samples. The dip slides confirmed that at the time the samples were taken, the bacterial load in the cooling tower water was 10^3 to 10^4 CFU/mL. The sulfamate data is shown in Table D.

Table D: Summary of the Size and Sulfamate Levels in Cooling Towers From a Chemical Manufacturing Plant

Cooling Tower	*Volume (gallons)*	*Sulfamate (ppm)*
Tower A	50,000	96
Tower B	35,000	216
Tower C	45,000	19
Tower D	16,000	207
Tower E	20,000	52

The sulfamate levels show that the cooling towers are under very different loads and that they fed differing amounts of stabilized bromine. Cooling towers had more than 50 and 90 ppm sulfamate and were still under acceptable microbial control. It might have been predicted that at these sulfamate levels, the cooling towers should be overstabilized. However, these real-world examples are consistent with the biofilm and planktonic data that were generated in this study. What was a bit surprising was that for two of these cooling towers, acceptable microbial control was achieved at sulfamate levels around 200 ppm. This is a much higher sulfamate level than would have been predicted for being able to maintain microbiological control.

Conclusions

The data from the planktonic bacteria testing showed that, while additional sulfamate reduced efficacy of the biocide, excellent log reductions were achieved at contact times relevant to a cooling tower, even with an excess of 100 ppm sulfamate present.

The data from the biofilm testing also showed that no change in the MBEC at up to 150 ppm added excess sulfamate.

Finally, the real-world data showed that cooling towers containing from 50 to more than 200 ppm sulfamate were still able to maintain good biological control when using stabilized bromine as the biocide.

All of this data (planktonic, biofilm, and real world) clearly shows that most of the speculation in the industry about at what sulfamate level overstabilization definitely occurs is too conservative. We suggest that there is not one particular sulfamate level above which overstabilization definitely occurs in all cooling towers. Variables like the type and amount of bacteria, organic load, presence of ammonia, and temperature will surely impact this value. Thus, the level of sulfamate that needs to be present for overstabilization to occur will depend on the conditions present in a cooling tower at a particular point in time.

Acknowledgements

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Endnote

[A] STABROM® 909 is the stabilized bromine biocide referenced in the text that was used in the experiments. It is manufactured by Albemarle Corp.

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