Comparative Efficacy of 21 ppm Br_2 from Three Different Sanitizers against *Salmonella typhimurium* and *Escherichia coli* O157:H7

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Purpose

The purpose of this study is to compare the efficacy of 21 ppm as Br_2 from three different Enviro Tech Chemical Services' products; BromMax® 7.1, bleach activated HB2®, and DBDMH against *Salmonella typhimurium* and *Escherichia coli* O157:H7.

Materials and Methods

Salmonella typhimurium (ATCC® 14028) was cultured in Sigma Nutrient broth at 35°C for 48 hours. The bacteria were separated from broth by centrifugation. The liquid was decanted and the bacterial pellet was reconstituted in 3.5L sterile phosphate buffer. The 3.5 L solution was then split into seven, 500mL samples. One sample was used as the control and the others served as the testing samples. An aliquot was removed from the control sample, serially diluted, and plated on 3M Enterobacteriaceae Petrifilms®.

Escherichia coli (ATCC® 35150) was grown in Sigma Nutrient broth at 35°C for 48 hours. The bacteria were separated from broth by centrifugation. The liquid was decanted and the bacterial pellet was reconstituted in 3.5L sterile phosphate buffer. The 3.5 L solution was then split into seven, 500mL samples. One sample was used as the control and the others served as the testing samples. An aliquot was removed and plated on 3M E. coli/Coliform Petrifilms®.

A 1% erythorbic acid solution was prepared by adding 1.0 gram of solid erythorbic acid to 99 grams of sterile deionized water.

<u>BromMax® 7.1 vs *Salmonella typhimurium*</u>

30 Second Contact Time

Prior to the addition of BromMax the pH of the solution was 7.47. The 500 mL test solution was dosed with 67µL of BromMax (Lot# 028L-262 7.04% Cl_2 15.84% Br_2). The solution was immediately analyzed for Br_2 using the Palin DPD methodology. The immediate recovery of Br_2 was 21.83 ppm. The pH of the solution after addition was 8.57. The solution was allowed to mix

for 30 seconds after which 1.22 mL of a 1% erythorbic acid was added to neutralize the remaining Br_2. An aliquot was removed and plated on 3M Enterobacteriaceae Petrifilms®.

60 Second Contact Time

Prior to the addition of BromMax the pH of the solution was 7.49. The 500 mL test solution was dosed with 66µL of BromMax (Lot# 028L-262 7.04% Cl_2 15.84% Br_2). The solution was immediately analyzed for Br_2 using the Palin DPD methodology. The immediate recovery of Br_2 was 19.58 ppm. The pH of the solution was 8.53. The solution was allowed to mix for 30 seconds after which 1.22 mL of a 1% erythorbic acid was added to neutralize the Br_2. An aliquot was removed and plated on 3M Enterobacteriaceae Petrifilms®.

Activated HB2® vs *Salmonella typhimurium*

30 Second Contact Time

A 3150 ppm Hypobromous acid stock solution was prepared by combining HB2® and sodium hypochlorite in water. The 500 mL test solution was dosed with a nominal 21 ppm Br_2 (3.33mL). Prior to the addition of the activated HB2® the pH was 7.58. Immediately after the addition of activated HB2® the pH was 7.47. An aliquot was removed and analyzed for Br_2 using the Palin DPD methodology and the immediate recovery was 20.93 ppm Br_2. After 30 seconds of mixing 1.22 mL of a 1% erythorbic acid was added to neutralize remaining Br_2. An aliquot was removed from the control sample, serially diluted, and plated on 3M Enterobacteriaceae Petrifilms®.

60 Second Contact Time

A 3150 ppm Hypobromous acid stock solution was prepared by combining HB2® and sodium hypochlorite in water. The 500 mL test solution was dosed with a nominal 21 ppm Br2 (3.33mL). Prior to the addition of the activated HB2® the pH was 7.60. Immediately after the addition of HB2® the pH was 7.46. An aliquot was removed and analyzed for Br_2 using the Palin DPD methodology and the immediate recovery was 19.13 ppm Br_2. After 60 seconds of mixing 1.22 mL of a 1% erythorbic acid was added to neutralize remaining Br_2. An aliquot was removed from the control sample, serially diluted, and plated on 3M Enterobacteriaceae Petrifilms®.

DBDMH vs *Salmonella typhimurium*

30 Second Contact Time

One gram of powder DBDMH was dissolved in 99 mL of soft water. The solution was allowed to mix for 10 minutes then the powder that did not dissolve in solution was removed by gravity

filtration. The DBDMH stock solution was analyzed using the Palin DPD methodology and the Br_2 concentration was 1087 ppm. The 500 mL test solution was dosed with a nominal 21 ppm Br_2 from DBDMH (9.66mL). Prior to the addition of DBDMH solution the pH was 7.56. Immediately after the addition of DBDMH the pH was 7.33. An aliquot was removed and analyzed for Br_2 using the Palin DPD methodology and the immediate recovery was 20.93 ppm Br_2. After 30 seconds of mixing 1.61 mL of a 1% erythorbic acid was added to neutralize remaining Br_2. An aliquot was removed from the control sample, serially diluted, and plated on 3M Enterobacteriaceae Petrifilms®.

60 Second Contact Time

The 500 mL test solution was dosed with a nominal 21 ppm Br_2 (9.66mL) from the stock solution. Prior to the addition of DBDMH the pH was 7.61. Immediately after the addition of DBDMH the pH was 7.49. An aliquot was removed and analyzed for Br_2 using the Palin DPD methodology and the immediate recovery was 19.13 ppm Br_2. After 60 seconds of mixing 1.61 mL of a 1% erythorbic acid was added to neutralize remaining Br_2. An aliquot was removed from the control sample, serially diluted, and plated on 3M Enterobacteriaceae Petrifilms®.

BromMax® 7.1 vs *Escherichia coli* O157:H7

30 Second Contact Time

Prior to the addition of BromMax the pH of the solution was 7.58. The 500 mL test solution was dosed with 66µL of BromMax® (Lot# 028L-262 15.84% Br_2). The solution was immediately analyzed for Br_2 using the Palin DPD methodology. The immediate recovery of Br_2 was 20.93 ppm. The pH of the solution was 8.48. The solution was allowed to mix for 30 seconds after which 1.22 mL of a 1% erythorbic acid was added to neutralize the Br_2. An aliquot was removed and plated on 3M E. coli/Coliform Petrifilms®.

60 Second Contact Time

Prior to the addition of BromMax® the pH of the solution was 7.56. The 500 mL test solution was dosed with 66µL of BromMax® (Lot# 028L-262 15.84% Br_2). The solution was immediately analyzed for Br_2 using the Palin DPD methodology. The immediate recovery of Br_2 was 18.68 ppm. The pH of the solution was 8.34. The solution was allowed to mix for 60 seconds after which 1.22 mL of a 1% erythorbic acid was added to neutralize the Br_2. An aliquot was removed, serially diluted and plated on 3M E. coli/Coliform Petrifilms®.

Activated HB2® vs *Escherichia coli* O157:H7

30 Second Contact Time

The 500 mL test solution was dosed with a nominal 21 ppm Br_2 (3.33mL) from the 3150 ppm Br_2 stock solution. Prior to the addition of activated HB2® the pH was 7.54. Immediately after the addition of activated HB2® the pH was 7.44. An aliquot was removed and analyzed for Br_2 using the Palin DPD methodology and the immediate recovery was 20.03 ppm Br_2. After 30 seconds of mixing 1.22 mL of a 1% erythorbic acid was added to neutralize remaining Br_2. An aliquot was removed, serially diluted and plated on 3M E. coli/Coliform Petrifilms®.

60 Second Contact Time

The 500 mL test solution was dosed with a nominal 21 ppm Br_2 (3.33mL) from the 3150 ppm Br_2 stock solution. Prior to the addition of activated HB2® the pH was 7.49. Immediately after the addition of HB2® the pH was 7.38. An aliquot was removed and analyzed for Br_2 using the Palin DPD methodology and the immediate recovery was 19.35 ppm Br_2. After 60 seconds of mixing 1.22 mL of a 1% erythorbic acid was added to neutralize remaining Br_2. An aliquot was removed, serially diluted and plated on 3M E. coli/Coliform Petrifilms®.

DBDMH vs *Escherichia coli* O157:H7

30 Second Contact Time

The 500 mL test solution was dosed with a nominal 21 ppm Br_2 (9.66mL) from the stock solution. Prior to the addition of DBDMH the pH was 7.50. Immediately after the addition of DBDMH the pH was 7.35. An aliquot was removed and analyzed for Br_2 using the Palin DPD methodology and the immediate recovery was 21.15 ppm Br_2. After 30 seconds of 1.61 mL of a 1% erythorbic acid was added to neutralize remaining Br_2. An aliquot was removed, serially diluted and plated on 3M E. coli/Coliform Petrifilms®.

60 Second Contact Time

The 500 mL test solution was dosed with a nominal 21 ppm Br_2 (9.66mL) from the stock solution. Prior to the addition of DBDMH the pH was 7.56. Immediately after the addition of DBDMH the pH was 7.49. An aliquot was removed and analyzed for Br_2 using the Palin DPD methodology and the immediate recovery was 20.25 ppm Br_2. After 60 seconds of mixing 1.61 mL of a 1% erythorbic acid was added to neutralize remaining Br_2. An aliquot was removed, serially diluted and plated on 3M E. coli/Coliform Petrifilms®.

Results and Discussion

Table 1 shows the initial pH, the pH after the addition of sanitizer, and the concentration of Br_2 recovered in solution

Description	Initial pH	Final pH	Br_2 Conc. (ppm)
Salmonella typhimurium			
BromMax® 7.1 30 sec Contact Time	7.47	8.57	21.83
BromMax® 7.1 60 sec Contact Time	7.49	8.53	19.58
HB2® 30 sec Contact Time	7.58	7.47	20.93
HB2® 60 sec Contact Time	7.60	7.46	19.13
DBDMH 30 sec Contact Time	7.56	7.33	20.93
DBDMH 60 sec Contact Time	7.61	7.49	20.03
Escherichia coli			
BromMax® 7.1 30 sec Contact Time	7.58	8.48	20.93
BromMax® 7.1 60 sec Contact Time	7.56	8.34	18.68
HB2® 30 sec Contact Time	7.54	7.44	20.03
HB2® 60 sec Contact Time	7.49	7.38	19.35
DBDMH 30 sec Contact Time	7.50	7.35	21.15
DBDMH 60 sec Contact Time	7.56	7.49	20.25

Table 2 shows the average *Salmonella typhimurium* counts in the control and test solutions.

Salmonella typhimurium

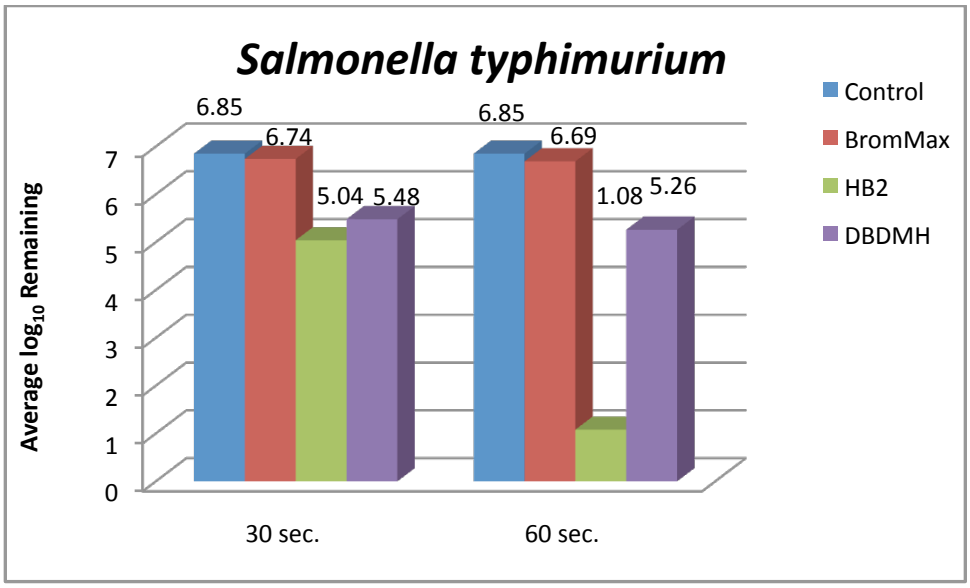
30 Second Contact Time

Description	Avg. log_{10}	Avg. log_{10} reduction	Percent Reduction
Control	6.85	NA	NA
BromMax® 7.1 30 sec	6.74	0.10	21
HB2® 30 sec	5.04	1.80	99
DBDMH 30 sec	5.48	1.37	96

60 Second Contact Time

Description	Avg. log_{10}	Avg. log_{10} reduction	Percent Reduction
Control	6.85	NA	NA
BromMax 60 sec	6.69	0.15	29
HB2® 60 sec	1.08	5.77	99.999
DBDMH 60 sec	5.26	1.59	97

Graph 1 is the graphical representation of the results from Table 2 for *Salmonella typhimurium* at a 30 and 60 second contact time.



The *Salmonella typhimurium* control had an average $\log_{10}$ of 6.85 CFU/mL. After treatment with 21 ppm Br_2 from BromMax® 7.1 for 30 seconds the remaining $\log_{10}$ was 6.74 CFU/mL. This is only a 0.10 CFU/mL reduction. After treatment with 21 ppm Br_2 from activated HB2® for 30 seconds the average remaining $\log_{10}$ of *S. typhimurium* was 5.04 CFU/mL which is a log reduction of 1.80 CFU/mL, or 99%. After treatment with 21 ppm Br2 from DBDMH for 30 seconds the average $\log_{10}$ of *S. typhimurium* decreased to 5.48 CFU/mL which equates to 96% reduction.

Treatment with 21 ppm Br_2 from BromMax® 7.1 for 60 seconds only decreased the $\log_{10}$ to 6.69 CFU/mL which is only a 29% reduction. Treatment with 21 ppm Br_2 from DBDMH for the same period decreased the average $\log_{10}$ to 5.26 CFU/mL which equates to a 97% reduction. A 60 seconds treatment with 21 ppm Br_2 from activated HB2® decreased the $\log_{10}$ to 1.08 CFU/mL which is a $\log_{10}$ reduction of 5.77 CFU/mL, or 99.999%.

Table 3 shows the average *Escherichia coli* O157:H7 counts in the control and test solutions.

Escherichia coli O157:H7

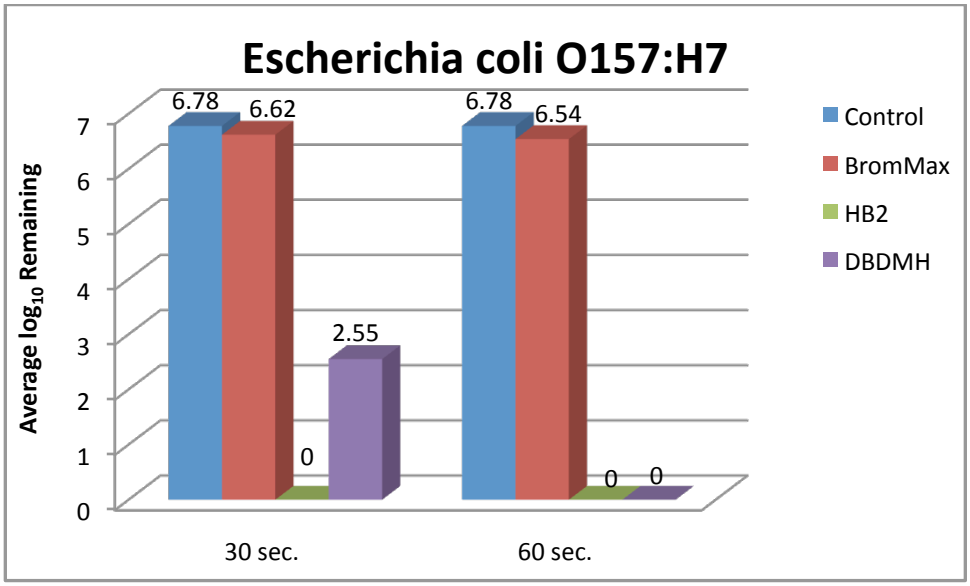
30 Second Contact Time

Description	Avg. $\log_{10}$	Avg. $\log_{10}$ reduction	Percent Reduction
Control	6.78	NA	NA
BromMax® 7.1 30 sec	6.62	0.15	30
HB2® 30 sec	0	6.78	>99.9999
DBDMH 30 sec	2.55	4.22	99.99

60 Second Contact Time

Description	Avg. $\log_{10}$	Avg. $\log_{10}$ reduction	Percent Reduction
Control	6.78	NA	NA
BromMax® 7.1 60 sec	6.54	0.23	42
HB2® 60 sec	0	6.78	>99.9999
DBDMH 60 sec	0	6.78	>99.9999

Graph 3 is the graphical representation of the results from Table 3 for *Escherichia coli* O157:H7 at a 30 and 60 second contact time.



The control for the *Escherichia coli* O157:H7 had an average $\log_{10}$ of 6.78 CFU/mL. After treatment with 21 ppm Br_2 from BromMax® 7.1 for 30 seconds the average $\log_{10}$ decreased slightly to 6.62 CFU/mL remaining. After treatment with 21 ppm Br_2 from activated HB2® for 30 seconds there were zero remaining *Escherichia coli* O157:H7 colonies. This is a $\log_{10}$ reduction of >6.78 CFU/mL which equates to a >99.9999% reduction. When treated with 21 ppm Br_2 from DBDMH for 30 seconds the average $\log_{10}$ decreased to 2.55 CFU/mL which is 99.99% reduction.

When treated with 21 ppm Br_2 from BromMax® 7.1 for 60 seconds the average log_{10} decreased minimally to 6.54 CFU/mL which is only a 42% reduction. After treatment with 21 ppm Br_2 from activated HB2® for 60 seconds there was zero remaining colonies which equates to a log_{10} reduction of >6.78CFU/mL or a >99.9999% reduction. When treated with 21 ppm Br2 from DBDMH there were zero remaining colonies and this equates to a >99.9999% reduction.

Conclusions

- This study clearly demonstrates that microbiological efficacy, and rate of efficacy in particular, is a function of the thermodynamic stability of the microbiocide. Although all 3 chemistries tested are sources of bromine, the rates at which these chemistries perform are quite different. For example, BromMax 7.1 is a highly stabilized sulfamic acid-complexed source of bromine which performed poorly over the short 30 seconds and 1 minute contact times compared to the other two sources. Bromine from DBDMH is stabilized to a lesser extent as a HOBr-DMH complex and so did not perform as rapidly or completely as unstabilized HOBr from activated HB2®.

- For *Salmonella typhimurium*, treatment with 21 ppm Br_2 from BromMax® 7.1 for one minute only decreased the log_{10} to 6.69 CFU/mL which is only a 29% reduction. Treatment with 21 ppm Br_2 from DBDMH for the same period decreased the average log_{10} to 5.26 CFU/mL which equates to a 97% reduction. A one minute treatment with 21 ppm Br_2 from activated HB2® decreased the log_{10} to 1.08 CFU/mL which is a log_{10} reduction of 5.77 CFU/mL which equates to a 99.999% reduction.

- The same trend is true for the *Escherichia coli* O157:H7. After treatment with 21 ppm Br_2 from BromMax® 7.1 the bacteria were reduced by only 31% compared to the control after 30 seconds. When treated with 21 ppm Br_2 from DBDMH for 30 seconds the average log_{10} decreased to 2.55 CFU/mL which is 99.99% reduction.

- Treatment with 21 ppm Br_2 from activated HB2® for 30 seconds there was zero remaining *Escherichia coli* O157:H7 colonies. This is a log_{10} reduction of >6.78 CFU/mL which equates to a >99.9999% reduction.

- The level and speed of kill of the HOBr from activated HB2® makes it the ideal chemistry for use in a poultry finishing chiller where short contact times for near total eradication of *Salmonella typhimurium* and other pathogenic bacteria are of vital importance to a poultry plant's HACCP program.