Efficacy of Hypobromous Acid from Sodium Hypochlorite-Activated HB2 against non-O157 Pathogenic E. coli

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Experimental Methods

Serovars Tested:

- E. coli O157:H7 (ATCC 35150)
- E. coli O26 (ATCC 12795)
- E. coli O103 (ATCC 23982)
- E. coli O45 (obtained from USDA-ARS-NPA, US Meat Animal Research Center, Clay Center, NB)
- E. coli O111 (ATCC 33780)
- E. coli O121(obtained from USDA-ARS-NPA, US Meat Animal Research Center, Clay Center, NB)
- E. coli O145 (obtained from USDA-ARS-NPA, US Meat Animal Research Center, Clay Center, NB)

Each of the above mentioned test systems were cultured in sterile nutrient broth (Sigma, St. Louis, MO) by incubation for two days at 35o C. The bacteria were separated from the nutrient broth by centrifugation and carefully resuspended in just over one liter of sterile phosphate buffer. The amount of E. coli bacteria was measured by serial dilution and plating onto 3M E. coli Petrifilms. Each solution was then split into two 500 ml samples. 0.5 ml of fetal bovine serum (FBS) was added to one of the two samples (0.1% FBS in solution).

HB2 activation was achieved by blending with a hypochlorite source. For this study, hypobromous acid was prepared by combining 24% aqueous hydrogen bromide (4.8 ml of HB2) and sodium hypochlorite (10.2 ml of 12.5% as Cl2 bleach) into 850 g of soft water to produce a stock solution of 3038 mg/L of HOBr (as Br2). One at a time, the 500 ml samples were treated with a nominal dose of 5 ppm of available bromine (as Br2) by introducing 0.83 ml of the 3038 mg/L (as Br2) stock solution. Immediately thereafter, the actual concentration of the test material was measured using the glycine modification of the DPD colorimetric method to confirm that all of the halogen present was HOBr. Approximately every two minutes, a calculated amount of the concentrated stock solution was added to each of the test solutions to replenish the HOBr that had been depleted. The HOBr concentration was tracked throughout a 20 minute time interval. After 2, 10 and 20 minutes a 20 ml aliquot was removed from the sample. The HOBr remaining in the 20 ml aliquot was neutralized by adding 0.31 g of a 0.1% erythorbic acid solution. The amount of viable E. coli bacteria remaining in the neutralized aliquot was

Background

On September 13, 2011 the U.S. Department of Agriculture announced that new steps will be taken to further safeguard the food supply in the USA. Six additional Shiga-toxin producing E. coli serogroups, or non-O157 STEC, will be declared adulterants if detected in raw ground beef, its components, or tenderized steaks. If any of the E. coli serogroups O26, O103, O45, O111, O121 and O145 are detected in raw ground beef, or precursor cuts, those products will be prohibited from sale to consumers. The FSIS will begin enforcing this new policy March 5, 2012.

Currently all establishments producing raw beef products are required to have one or more critical control points in their HACCP plans to eliminate or to reduce E. coli O157:H7 below detectable levels. With this new regulation, these establishments will need to reassess their HACCP plans to include the six non-O157 E. coli serogroups. Previous studies performed by Enviro Tech Chemical Services demonstrate that hypobromous acid (from activated HB2TM) is efficacious against E. coli O157:H7, but the purpose of this study is to observe the sensitivity of these six serogroups compared to E. coli O157:H7 by using a low concentration of the antimicrobial. A low level of the activated hypobromous acid was chosen in this challenge test to allow the investigators to observe and track the relative efficacy of each serovar over time in the presence of the oxidizer.

measured by serial dilution and plating on 3M E. coli Petrifilms. All the Petrifilms were incubated at 35o C for 24 hours before enumeration and comparison with the untreated control (plated previous to splitting into two 500 ml samples and challenging with HB2-activated HOBr).

This study was performed in duplicate on two separate days, and the average of the results for all plating and challenges are is reported below. In addition, all challenge testing was performed at pH values of 6.8-7.2.

Results and Discussion

There was no significant difference between samples that contained FBS and those that did not; as a result, only the data for the samples containing the FBS (organic matter) is reported below.

During the challenge testing, the residual HOBr (available bromine as Br2) was measured initially and at the 2, 10, 15 and 20 minute time intervals. This data is reported in Table 1. The Br2 concentration steadily declined throughout the test period and additional concentrated stock solution was added to replenish the Br2 that had been consumed.

TABLE 1: PPM of Oxidant: Initial (5 mg/L as $Br_{2)}$ nominal dose of HOBr followed by tracking the depletion with time and level following replenishment

Time	E. coli O157:H7 /mg/L as Br_2	E. coli O26 /mg/L as Br_2	E. coli O103 /mg/L as Br_2	E. coli O45 /mg/L as Br_2	E. coli O111 /mg/L as Br_2	E. coli O121 /mg/L as Br_2	E. coli O145 /mg/L as Br_2
Initial	5.85	4.32	4.59	5.18	2.88	5.18	4.73
2 min	4.72	4.19	3.47	2.25	5.64	4.05	3.38
10 min	1.80	4.19	3.73	4.06	3.47	5.85	5.18
15 min	5.18	3.65	3.56	4.50	6.48	3.83	3.15
20 min	2.48	4.23	4.50	4.95	5.63	4.04	6.08

Table 2 shows the remaining log10 CFU/ml of E. coli O157:H7 and the six non-O157 E. coli samples before treatment with, and maintenance to around 5 mg/L asBr_2, at 2, 10, and 20 minutes.

TABLE 2: Log10 CFU/ml E. coli serogroups remaining in solution over time

Time	E. coli O157:H7 /Log10 CFU /ml	E. coli O26 /Log10 CFU /ml	E. coli O103 /Log10 CFU /ml	E. coli O45 /Log10 CFU /ml	E. coli O111 /Log10 CFU /ml	E. coli O121 /Log10 CFU /ml	E. coli O145 /Log10 CFU /ml
Control	7.15	8.51	6.46	7.20	5.84	6.85	6.54
2 min	4.01	1.48	0.00	3.14	0.00	2.21	3.28
10 min	1.53	0.00	0.00	3.11	0.00	2.45	1.23
20 min	1.30	0.00	0.00	1.39	0.00	0.00	0.60

The log_{10} reduction, the reciprocal of the CFU's remaining, in comparison to the untreated control of each serogroup can be seen in Table 3.

TABLE 3: Log10 reduction of the E. coli serogroups over time

Time	E. coli O157:H7 /Log10/ml Reduction	E. coli O26 /Log10/ml Reduction	E. coli O103 /Log10/ml Reduction	E. coli O45 /Log10/ml Reduction	E. coli O111 /Log10/ml Reduction	E. coli O121 /Log10/ml Reduction	E. coli O145 /Log10/ml Reduction
2 min	3.14	7.03	>6.46	4.06	>5.84	4.64	3.26
10 min	5.62	>8.51	>6.46	4.09	>5.84 >	4.40	5.31
20 min	5.85	>8.51	>6.46	5.81	>5.84	>6.85	5.94

It can be seen that two of the serogroups (O103 and O111) were eradicated at or before the two minute contact time and one serogroup (O26) was eradicated at or before the ten minute interval after dosing with HOBr. The serogroup O121 was the next most sensitive to HOBr (5 mg/L as Br2) followed by O145 while O45 and O157:H7 proved to be the most resistant to this dose after 20 minutes of contact time.

Thus, in decreasing sensitivity to a dose of 5 mg/L as Br2 over a span of 20 minutes, the order is:

O103 ≈ O111 < O26 < O121 < O145 < O157:H7 ≈ O45

Conclusions

- The R&D department of Enviro Tech Chemical Services has performed several efficacy studies using HOBr (from activated HB2) against E. coli O157:H7 for meat processing facilities and FCN applications. This new regulation going into effect March 2012 has sparked the interest of several of these facilities who are wondering whether antimicrobials are efficacious against these six serogroups, and what is the comparison to E. coli O157:H7. Therefore, the purpose of this study was to determine the sensitivity of these six serogroups compared to E. coli O157:H7 by using a low concentration of HOBr (from activated HB2).
- Two of the serogroups (O103 and O111) were eradicated at or before the two-minute contact time
- One serogroup (O26) was eradicated at or before the ten-minute after dosing with HOBr. The serogroup O121 was the next most sensitive to HOBr (5 mg/L as Br2) followed by O145 while O45 and O157:H7 proved to be the most resistant to this dose after 20 minutes of contact time.
- Thus, in decreasing sensitivity to a dose of 5 mg/L as Br2 over a span of 20 minutes, the order is:

  O103 ≈ O111 < O26 < O121 < O145 < O157:H7 ≈ O45
- These results can be considered reliable and accurate for systems utilizing the bleach- activated HBr chemistry that is currently patent pending by the current authors, and cannot be assumed to be equal for any other alternate methodologies.